NONHUMAN PRIMATES IN BIOMEDICAL RESEARCH

Biology and Management

EDITORS:
B. Taylor Bennett, Christian R. Abee, Roy Henrickson

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Nonhuman Primates in Biomedical Research
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Biology and Management

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Preface

Nonhuman Primates in Biomedical Research represents the first definitive reference source for those who work with and care for nonhuman primates in biomedical research. Prior to the development of this work, available information was contained in a variety of journals, species-specific reference books, and in the minds of a limited number of experienced primatologists and veterinarians specializing in the care of nonhuman primates. This lack of a central information source was compounded by the fact that very few laboratory animal veterinarians had training and/or experience in managing and caring for these very unique and valuable animals. To meet this need, the Board of Directors of the American College of Laboratory Animal Medicine voted to add these books on nonhuman primates to the college's collection of published texts.

This volume provides basic information on the biology and management of the commonly used primate species in a format that can be used by veterinarians or nonveterinarians with the day-to-day responsibility for the care and use of these animals. Thus it serves as a general reference for those who care for and use nonhuman primates in biomedical research.

Since the maintenance of large populations of primates for biomedical research is limited to relatively few institutions, those with the prerequisite experience to author the various chapters were somewhat limited. Additionally, the number of animals available for study was very small compared to the number of other commonly used laboratory animals. The size of study populations is reflected in the small number of available publications on a subject or condition. This lack of published information presented a formidable challenge in producing this addition to the ACLAM series and served to emphasize the need for consolidating the information in a single reference source. To meet this challenge, it was necessary to seek authors whose areas of expertise covered a broad range of topics and whose careers had focused on the care and study of primates. Hence, this volume represents the work of a variety of scientists drawn from within our college, from the ranks of primate clinicians, and from the field of primatology. The authors relied heavily on their experience and extrapolation from the human and veterinary medical literature. We extend our sincere appreciation to this diverse group without whom this work would not have been possible.

While the authors are the heart and soul of a volume such as this, the reviewers are the conscience. They serve to provide an overview and to focus our attention which may have been obscured by familiarity with a task.

As for all volumes of the ACLAM series, the editors and authors have served without compensation, and have donated all publication royalties to the American College of Laboratory Animal Medicine to continue the work for which it was founded in 1957: to encourage education, training, and research in laboratory animal medicine and to recognize veterinary medical specialists in the field by certification and other means. We wish to express our appreciation to the officers and members of the college for their support and assistance during the evolution of this project.

We would like to acknowledge an author whose death during this project cost our community a respected and valued colleague, Dr. Benjamin Blood. His knowledge of the field and historical perspective will be missed.

B. TAYLOR BENNETT
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CHAPTER 1

History

Dennis O. Johnsen

I. HUMAN AND NONHUMAN PRIMATES TO 1960

A. Roots of Modern Primatology

Nonhuman primates probably first became valuable to man as pets, but they are also the oldest recorded animal subjects for scientific research (Hill, 1977). Nonhuman primate pet trading is known to have occurred in Egypt as long as 5000 years ago (Morris and Morris, 1966); their use for medical purposes came somewhat later, although still in respectably ancient times. Galen (130–200 AD) did anatomical studies on animals including monkeys (Cohen and Loew, 1984), and Vesalius (1514–1564) used barbary apes (*Macaca sylvanus*) in his studies of circulatory anatomy (Kavanaugh, 1984; Morris and Morris, 1966; Loeb *et al.*, 1989). Ruch (1941) has documented that monkeys and apes were studied from ancient times through the middle ages by Hanno, Aristotle, Pliny the Elder, Pliny the Younger, Petrus, Candidus, and others (Morris and Morris, 1966; Loeb *et al.*, 1989).

Darwin's research on evolution, and particularly his notes on the behavior of the gorilla, established his credentials as one of

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the first observational primatologists (Darwin, 1871). Also late in the 19th century, the British physician David Ferrier conducted comparative neuroanatomy studies of apes and monkeys (Morris and Morris, 1966). During this same time period, Pasteur discovered that rabies virus passaged through monkeys lost its virulence for dogs (Pasteur *et al.*, 1884a,b). Some 20 years later, the poliovirus was isolated by inoculating spinal cord material collected from fatal human cases intraperitoneally into monkeys (Landsteiner and Popper, 1908, 1909).

The primatological knowledge that was generated came largely from relatively few behavioral and biomedical investigators working independently. With the institutionalization of nonhuman primate research, a profound change became possible.

B. First Primate Centers

1. Soviet Institute of Experimental Pathology and Therapy

According to Held and Gay (1983) and Lapin (1983), the first Commissar of Health in the USSR was persuaded by Mechnikov, a pioneer of modern Soviet experimental primatology, to establish a primate breeding station in 1923. Located in Sukhumi on the subtropical shores of the Black Sea in the then Soviet State of Georgia, the station was intended to be a quarantine, breeding, and holding center for nonhuman primates and support a network of 50 medical and biomedical research institutions. It began operations in 1927 when it received the first shipment of hamadryas baboons (*Papio hamadryas*) and chimpanzees (*Pan troglodytes*) from Africa. At first, captive breeding was unsuccessful. However, there was improvement as experience in maintaining and breeding nonhuman primates was gained. Charting a course that has been followed elsewhere, activities of the Sukhumi station's service gradually expanded to encompass initiatives in independent research. In 1957, now under the auspices of the Academy of Medical Sciences of the USSR, the station became the Institute of Experimental Pathology and Therapy (IEPT) in recognition of its status as a full-fledged research institution. By 1990, IEPT had production colonies of over 7000 animals consisting primarily of baboon and macaque species, a staff of about 1000 people, and research programs focusing on oncology, physiology, biochemistry, infectious diseases, and the biology of nonhuman primates (B. A. Lapin, personal communication, 1990). The institute also served as a principal source of nonhuman primates for the Virology Institute in Moscow and the Russian space program and served as an international resource as well with productive research links to medical scientists in the United States and elsewhere.

The secession of Georgia from the Soviet Union and the disturbances associated with the declaration of independence of Abkhazia seriously disrupted operations in Sukhumi. These problems forced completion in 1992 of a move of less than 100 miles to a satellite site in Russia near the city of Adler (D. M. Bowden, personal communication, 1993). Despite this adversity, the institute, now the Institute of Medical Primatology of the Russian Academy of Medical Sciences, remains one of the largest nonhuman primate research centers in the world.

2. Robert Yerkes and the Primate Laboratory of the Yale Institute of Psychobiology

Robert Yerkes, an accomplished comparative psychologist, had a vision of what the future held for nonhuman primate research and how to realize those dreams (Yerkes, 1916). Yerkes established the Primate Laboratory of the Yale Institute of Psychobiology at Orange Park, Florida, in 1930 (Bourne, 1971; Maple, 1979). His plan was to establish and develop "an institute of comparative psychobiology in which the resources of the various natural sciences should be used effectively for the solution of varied problems of life" (Yerkes, 1932). As early as 1919, he proposed the idea of establishing a nonhuman primate research institute for the systematic study of the "fundamental instincts" and "social relations" of nonhuman primates. Yerkes was a contemporary of other notable early investigators of the time such as Kohler and Kohts who were interested in nonhuman primate research (Maple, 1979). Interest in Kohts' perceptual and sensory work with chimpanzees in the Soviet Union may have contributed to the initiative for establishment of the Sukhumi station (Yerkes, 1943).

Yerkes established his Orange Park station in 1930 with funds from Yale University and the Rockefeller and Carnegie Foundations. He received an initial gift of 13 chimpanzees from a breeding facility belonging to Rosalia Abreu in Cuba (Maple, 1979). The colony was expanded during the next several years with 16 additional chimpanzees from Africa, a gift from the Pasteur Institute. Laboratory studies were multicategorical, encompassing neurophysiology, anatomy, pathology, nutrition, growth, and development (Bourne, 1971). The station set an early, if not the first, organizational precedent for a comprehensive nonhuman primate research center.

In 1965, the laboratories in Orange Park were moved to Atlanta, Georgia, and the animals were reestablished in the new Yerkes Regional Primate Research Center of Emory University.

3. Cayo Santiago Colony

Clarence Ray Carpenter, a student of Yerkes and an accomplished field primatologist (Maple, 1979), has as one of his most enduring accomplishments the establishment of the Cayo Santiago Colony of rhesus monkeys. Rawlins and Kessler (1986) and Kessler (1989) have provided extensive accounts of the history of the Cayo Santiago Colony. Much of the historical information cited next is derived from those accounts.

Carpenter formulated plans in the early 1930s for establishing a population of both gibbons and rhesus macaques on an island in the American tropics. The possibility of conducting both behavioral and biomedical research on an island colony

was basic to those plans. He interested a number of people, including the staff of Harvard's Museum of Comparative Zoology, the faculty of Columbia University's College of Physicians and Surgeons, and the Columbia University/University of Puerto Rico's School of Tropical Medicine (later to become a component of the University of Puerto Rico School of Medicine), in the planning effort. He selected Cayo Santiago, a 15.2-ha (approximately 38 acres) island 1 mile off Puerto Rico's eastern coastal town of Humacao, as the site for the colony.

With the help of a $60,000 grant from a private foundation, Carpenter set off for Indochina and India in 1938. He fared well in collecting rhesus monkeys but not gibbons. Survival of the 47-day voyage from Calcutta as deck cargo was a testimonial to the enduring qualities of the rhesus monkeys and the care that they received. By early 1939, 409 rhesus monkeys, 14 gibbons, and 3 pig-tailed macaques were released on Cayo Santiago. Eventually only the rhesus monkeys remained.

Maintenance of the island and breeding were not without problems. Local fruits and vegetables did not provide an adequate diet and malnutrition was overcome only by feeding fox chow, the early precursor to monkey chow. Wells were dug, but the water was brackish. Cisterns and a system for collecting rainwater had to be constructed. A number of monkeys were lost through fighting or simply through being crowded out in the establishment of a stable social structure. Some escaped by swimming the channel to the mainland. Various diseases also took their toll and there were few opportunities to limit spread. However, persistent efforts did finally result in eliminating tuberculosis.

Another problem was the lack of dependable financial support for the project. In 1944, the University of Puerto Rico assumed full responsibility for support of the colony. Operations languished until 1948 when efforts to attract badly needed outside support were successful. At that time, the National Institutes of Health (NIH) awarded the first of a number of federal awards to the University of Puerto Rico to help support Cayo Santiago. Eventually, the island operation was incorporated as a component of the University's Caribbean Primate Research Center.

Cayo Santiago has been a valuable resource through the years for both the production of monkeys and for biomedical and behavioral research. The experiences with Cayo Santiago have also shown that outside support, primarily federal government support, is essential for the long-term maintenance of nonhuman primate resources.

C. Virological Research and Nonhuman Primates

Technically, the modern use of nonhuman primates in biomedical research had its origins in Pasteur's work with rabies and the studies of others with smallpox and vaccinia in the late 1800s. Kalter and Heberling (1971) and Gerone (1974) have provided comprehensive reviews of virological research in nonhuman primates, including work on yellow fever and a variety of encephalitis viruses through the 1930s.

The Nobel prizewinning achievement of Landsteiner and Popper in isolating poliovirus in Vienna (1908, 1909) provided the real beginning of serious and widespread use of nonhuman primates in biomedical research, using rhesus monkeys, baboons, and chimpanzees in their work. The unique susceptibility of nonhuman primates to a relatively new and frightening disease threat clearly established their special importance in research.

The intense efforts to develop a vaccine against polio that followed was unprecedented. It spanned the next 45 years, was international in scope, and involved a host of major investigators. However, it was a complex process that experienced serious setbacks. There were some promising early findings based on nonhuman primate studies using inactivated, or partially inactivated, vaccines. However, those findings led to disastrous results when cases of paralytic polio occurred following vaccinations in human clinical trials (Horstmann, 1985).

Nevertheless, nonhuman primates played an important role in helping to put polio research back on track. In 1931, throat washings from patients were inoculated into monkeys and resulted in infection (Paul and Trask, 1932). Later work showed that poliomyelitis was an enteric and not an olfactory infection. The discovery by Enders and co-workers (1949) that poliovirus could be grown in human tissue culture was a major scientific advance which brought them the Nobel Prize for Medicine in 1954. It also provided a means to reduce the need for nonhuman primates. However, like the promise computers initially offered for reduction of paperwork, any reduction in the use of nonhuman primates was soon masked by a vastly expanded and accelerated research effort which required even more animals.

Salk's report of a formalin-inactivated polio vaccine grown in monkey kidney cell culture paved the way for extensive and successful field trials (Salk *et al.*, 1953). Unfortunately, this dramatic achievement was clouded by uncertainty when improperly inactivated vaccine caused a number of cases of polio in 1955 (Horstmann, 1985).

At about the same time, Sabin was working on the development of a polio vaccine from another direction. Depending greatly on the use of monkeys and chimpanzees, he searched for attenuated strains of naturally occurring poliovirus. His painstaking work reportedly used 9000 monkeys and 150 chimpanzees (Sabin, 1985). The result was the development of an oral polio vaccine that remains in widest use today.

While relatively modest in the early years, the use of monkeys increased dramatically following Salk's discovery of an effective vaccine. The high point of this usage was in 1957 and 1958 when about 200,000 monkeys were imported annually into the United States (Lecornu and Rowan, 1979). According to Lecornu and Rowan, the greatest single use of the more than 1.2 million rhesus monkeys that were imported into the United States during the 20 years that followed Salk's discovery was for producing and testing polio vaccine.

The legacy of the 1955 polio vaccine incident continues even today. The more rigorous testing program that was adopted after the incident accounts for 20–25% of all nonhuman primates used in research and testing (Marten, 1981). However, the number of animals required for testing polio vaccine has declined dramatically. Polio ushered in an era of sponsored research that was necessary for the development of domestic nonhuman primate resources. It also firmly established nonhuman primates in the public eye as research animals rather than curiosities.

D. Other Contributions

1. Work of Harry Harlow

Harry Harlow started his studies on the learning abilities of monkeys in 1930 at the University of Wisconsin. After conducting comparative studies of learning capabilities of cebus and rhesus monkeys at the local Vilas Park Zoo, he developed a modest laboratory on the university campus. During a career spanning nearly 50 years, Harlow expanded these resources into a large and interdisciplinary research complex that included the Wisconsin Regional Primate Research Center (Davenport, 1979). This research enterprise eventually had a staff of over 200 people and a nonhuman primate colony numbering in excess of 1000 monkeys.

Harlow shed light on the nature and limits of rhesus monkey intelligence. Studies in the infant monkey nursery focused on the results of enriched versus impoverished social rearing conditions, development of measures of learning ability, and surrogate-mother testing to demonstrate the importance of infant–maternal tactile sensations relative to biological drives such as hunger. His work opened new areas of study including nonhuman primate emotions such as love, parent–child relationships, peer interaction, play, heterosexual behavior, and psychological impairments that result from social deprivation and separation (Suomi and Leroy, 1982).

Attempts have been made to discredit Harlow's work as cruel and trivial. On the contrary, Harlow's research provided insight into nonhuman primate well-being, captive breeding, rearing, and maintenance. This important work continues to have a major influence on both nonhuman primate and human behavioral research.

2. Breeding and Reproductive Physiology

Surprisingly little information on the subject of nonhuman primate reproductive physiology and breeding prior to the 1960s exists. The first chimpanzee, or any ape for that matter, was not born in captivity until 1915 (Montane, 1915). As late as 1938, Carl Hartman, a prominent reproductive physiologist, predicted that rhesus monkeys would not breed in the American tropics (Rawlins and Kessler, 1986).

Gertrude van Wagenen, a faculty member in the Department of Obstetrics and Gynecology at Yale University School of Medicine, may have been the first to establish a captive rhesus monkey laboratory breeding colony (van Wagenen, 1972; D. M. Horstmann, personal communication, 1989). Over a career spanning 45 years, she collected detailed information from birth to death on all of the 1261 monkeys that lived in the colony. The colony produced 600 live births through 15 generations. Her many publications provided an abundance of basic information on rhesus reproduction and rearing. This work represents one of the first major efforts to focus on characterizing this facet of rhesus monkey biology. Studies of monkey biology, as an end in itself, did not come until later.

3. Herpesvirus B and Other Biohazards

Until relatively recently, people did not appreciate that there was much to fear from nonhuman primates except physical injury. Tuberculosis was recognized fairly early as a relatively common disease in humans. However, it was more devastating to nonhuman primates than it was to man. It was not until 1934 that serious concerns arose about other biohazards in nonhuman primates. In that year, a fatal human case of encephalitis caused by the herpesvirus B occured following a monkey bite (Sabin and Wright, 1934).

Since 1934, there have been more than 20 fatal cases of human infections with herpesvirus B (Palmer, 1987). There have been few survivors among these cases, although the use of acyclovir now appears to hold infection in abeyance. Hull (1973) reported earlier that there had been at least 83 cases of human diseases caused by simian viruses, including 23 deaths. In addition to herpesvirus B, hepatitis virus (Hillis, 1961) and Marburg virus (Kissling *et al.*, 1968) represent two of the more important diseases. Concern about these diseases and the precautions that laboratory personnel must take when working with the nonhuman primates placed these animals in a very special class among laboratory animals.

II. ESTABLISHMENT OF PRIMATE CENTERS: CROSSING THE THRESHOLD

A. Initial Activity

The extended process that led to the establishment of the NIH Regional Primate Research Centers Program (RPRCP) has been well documented (Anonymous, 1968). It dates back to 1947 and 1949 when NIH unsuccessfully tried to establish a procurement program to make an adequate supply of chimpanzees available to researchers in the United States.

In the period from 1955 to 1957, a number of groups and individuals advising NIH and the National Academy of Sciences–National Research Council noted the need for developing additional nonhuman primate research facilities. Not much happened until the director of the NIH National Heart Institute

(NHI), and eventually the director of NIH, became interested in the problem.

B. Developing the Concept

In 1956, Karl F. Meyer, initially trained as a veterinarian and known to the world of science for his research in microbiology and directorship of the University of California at San Francisco's Hooper Institute, visited the Sukhumi station in the USSR. On his return, he urged the NIH Director, James Shannon, to develop a nonhuman primate research colony in the United States. In the same year, NHI Director James Watt also visited Sukhumi because of the work in experimental hypertension being done there using nonhuman primates.

Watt's report led the advisory council of the NHI in 1957 to recommend the development of a nonhuman primate colony associated with a university to serve as a site for a long-term multidisciplinary approach to research on cardiovascular problems. Shannon had differing views about the wisdom of establishing a single station with a focus limited to long-term cardiovascular research.

In late 1958, the NHI concluded that a nonhuman primate station was both feasible and desirable. With increased interest in this idea within Congress, NHI began to plan for a station. Conspicuous in this planning effort were George Burch, a noted cardiovascular researcher from Tulane University, and Willard "Hal" Eyestone. Eyestone was the responsible NHI staff officer who would eventually become first director of the new regional nonhuman primate research centers program.

Congress received the planning report on NIH's plans for a nonhuman primate program in mid-1959. This plan reflected a transition in thinking about a single station, as conceived by NHI, to a number of smaller nonhuman primate research centers. These centers were still to focus on cardiovascular research, but their roles were expected to expand to include "other disease categories and other disciplines, until ultimately the functions of the stations or centers is the full and complete investigation of the primate" (Anonymous, 1968).

The NIH planners felt that the focus of the centers should be on research, not just serving as a source of monkeys, and that support should be provided by NIH for a long period of time. Fifty to 100 years was originally suggested. Other ideas also became cornerstones of the new program. Research was to be conducted on nonhuman primates in conjunction with other basic and clinical studies. Investigations were to be carried out on the usefulness of various species of nonhuman primates in research. A national reservoir of information on nonhuman primates and for nonhuman primate research was to be provided. There were to be facilities for visiting scientists and research training. Extensive local participation with appropriate universities or research institutions and the need for seeking outside funding to augment the core budget were also identified as basic concepts.

C. Launching the New Program

Congress appropriated the first funding for the program, $2 million, in 1959. There were to be several centers. These centers were to be geographically distributed, be part of a university environment, and support biomedical and health research broadly instead of being limited to a particular area such as cardiovascular research.

Following announcement of the new program in January, 1960, NHI received 11 applications. Seven applications were approved by the study section which reviewed the applications. NHI awarded the first grant to establish the Oregon Regional Primate Research Center at Beaverton, Oregon.

With a congressional appropriation of $7 million for the following year (FY 1961), NHI awarded grants to establish the Washington Regional Primate Research Center (RPRC) at the University of Washington in Seattle; the Wisconsin RPRC at the University of Wisconsin in Madison; the Yerkes RPRC in Atlanta in association with Emory University; the Delta RPRC in association with Tulane University at Covington, Louisiana (now the Tulane RPRC); and the New England RPRC in association with Harvard University at Southboro, Massachusetts.

Still preoccupied with the perceived need for a national "station," the advisory council of the NHI continued to urge the establishment of a conditioning center for nonhuman primates. Its function was to be the development of techniques for procuring, conditioning, and maintaining various nonhuman primates for study. In 1962, NHI awarded a grant to establish such a center at the University of California, Davis. The center, initially designated as the "National Center for Primate Biology," later became the California RPRC. This change was made after it became apparent that it was much more important and realistic to have the California center function as a RPRC rather than serving the specialized role originally envisaged.

By the time the initial establishment of the seven centers was complete in 1968, the 8 years of cumulative federal funding provided by NIH totaled about $52 million, including funds for the purchase of land sites, construction of the centers' facilities, other start-up costs, and a rapidly expanding research program. Administration of the Regional Primate Research Centers Program was formally transferred in 1962 to NIH's Division of Research Facilities and Resources. This division later became the NIH Division of Research Resources (DRR) and, in 1990, the National Center for Research Resources (NCRR).

D. Regional Primate Research Centers Program Today

By the end of 1991, the seven RPRCs had 199 core staff scientists, 835 collaborators and affiliates, 34 visiting scientists, and a total of about 17,000 nonhuman primates representing 32 different species; their breeding colonies produced about 2400 live births annually (L. H. Whitehair, personal communication, 1991). The program has been very successful. The seven cen-

ters have played a pioneering role, in which multidisciplinary interactions among veterinarians, reproductive physiologists, and behaviorists were crucial in developing techniques for the large-scale captive breeding of macaques. Fridman (1972) noted that the number of scientific publications based on research using nonhuman primates trebled in the 4- to 5-year period following 1964. He pointed out that the temporal relationship of this phenomena to the establishment of the centers was not accidental.

III. 1960–1980: PERIOD OF GROWTH IN A WORLD OF INCREASING CONSTRAINTS

A. Emulation of the Center Concept

1. General

By 1972, there were 40 research centers in the world devoted to experiments with nonhuman primates and another 1800 institutions were using nonhuman primates in research (Fridman, 1972). Fridman described growth in the field as "explosive." Between 1965 and 1971, the number of research projects using nonhuman primates increased from 666 to 1183 in the United States, an 80% increase (Goodwin, 1975a). Referring to data provided by the Primate Information Center of the Washington RPRC, Goodwin reported that the 5000 nonhuman primate references on record in 1960 had increased sevenfold to 35,000 by 1971. The status, usage, and availability of nonhuman primates in the United States during this period have been extensively reviewed by the Institute of Laboratory Animal Resources (Southwick, 1975).

2. Southwest Foundation for Research and Education

The Southwest Foundation for Research and Education (SFRE) was established in San Antonio, Texas, in 1941 and it first obtained baboons in 1957 (Vagtborg, 1973). In 1958 the NIH made a grant award to SFRE to support the development and operation of a baboon colony and this support continued until 1972. In addition to the research and production colony at SFRE, funding also provided support for conditioning and trapping facilities in Kenya and numerous baseline studies on the baboon. The success of the husbandry and baseline studies established the baboon as a nonhuman primate model for many areas of biomedical research and the SFRE as a leading baboon research center.

A reference center for nonhuman primate viruses was established at SFRE in 1965 and was designated as the NIH–World Health Organization's (WHO) Simian Viruses Reference Center in 1968 (Kalter and Heberling, 1971; Kalter, 1974). In 1982, a major research and diagnostic *Herpesvirus simiae* (B virus) program was established (Hilliard *et al.*, 1986). This program serves as the primary B virus resource in the United States and elsewhere. In 1977, the NIH awarded a grant to SFRE for developing and operating a semifree-ranging national baboon breeding program. This resource continues to provide baboons to investigators throughout the United States (W. J. Goodwin, personal communication, 1993).

In 1983 the SFRE became the Southwest Foundation for Biomedical Research.

3. Bowman Gray School of Medicine

This facility, a part of Wake Forest University in Winston-Salem, North Carolina, was established at roughly the same time as the RPRC program. Thomas Clarkson played a leading role in the development of this facility beginning in the late 1950s and he helped to firmly establish the subspecialty of nonhuman primate medicine in his profession and in biomedical science. With its contributions to cardiovascular research and the influence that veterinarians trained unter the tutelage of Clarkson have had, this facility also became a major nonhuman primate center.

4. Duke University Primate Center

The Duke University Primate Center in North Carolina began with a small colony obtained from Madagascar in 1960. Izard (1989) related that, with NIH and National Science Foundation (NSF) support, this colony was moved in 1966 from Yale University to its present location. It has continued to expand through breeding and acquisitions and now contains the largest collection of prosimians in the world. Varied amounts of financial support have been provided for the center by Duke University, NIH, NSF, and other sponsors. The future activities of this unique center have been jeopardized on several occasions by uncertain funding. Prosimians have not been widely used as animals for biomedical research and the center adheres to a noninvasive research policy because of their endangered status.

5. Laboratory for Experimental Medicine and Surgery in Primates

The Laboratory for Experimental Medicine and Surgery in Primates (LEMSIP), associated with New York University, began to serve the metropolitan New York City needs for a nonhuman primate center in 1965 (Anonymous, 1988). Jan Moor-Jankowski, a physician and immunologist, came to New York in that year to work with Alexander Wiener. Wiener, a hematologist, in earlier work with Landsteiner (Landsteiner and Wiener, 1940), had described the Rh factor in experiments using rhesus monkeys. They cooperated with other New York City area investigators by providing nonhuman primate resources. By late 1966, 23 local investigators had already taken advantage of this opportunity.

The LEMSIP is currently located at Sterling Forest, a New York City suburb. It is known for its early innovations in non-

human primate husbandry, chimpanzee breeding, research in hepatitis, and, most recently, AIDS. The LEMSIP was designated a WHO Collaborating Center for its preeminence in the field of nonhuman primate blood grouping.

6. Caribbean Primate Research Center

The establishment of the Caribbean Primate Research Center (CPRC) in Puerto Rico in 1970 represents a continuation of the Cayo Santiago story. Goodwin (1989) and Frontera (1989) have provided extensive background on this subject. In the late 1950s, the National Institute of Neurological Diseases and Stroke (NINDS) of the NIH, later named the National Institute of Neurological, Communicative Disorders and Stroke, negotiated with the University of Puerto Rico to establish the Laboratory of Perinatal Physiology (LPP). Part of this agreement included access to supplies of monkeys from Cayo Santiago. In 1970, the NINDS decided to discontinue the LPP, and its facilities, including animals, reverted to the university. Negotiations with the university at this time probably convinced NINDS, and possibly other NIH institutes, of the merit of maintaining these nonhuman primate resources. An initial contract for $300,000 was awarded to provide core support for a new center. Responsibility for the contract was transferred to the DRR of the NIH in 1972 and core support for the center has continued since that time.

7. Department of Defense Laboratories

Department of Defense (DOD) laboratories have contributed significant knowledge and the effort of many people to the field of medical primatology. Notable in this respect were the Army's laboratories at Edgewood Arsenal, Fort Detrick, Walter Reed Army Institute of Research, Air Force School of Aerospace Medicine, the Balcones Laboratory at the University of Texas in Austin, and the Aeromedical Laboratory facilities at Wright-Patterson and Holloman Air Force bases. In 1974, DOD laboratories had a total of 2600 nonhuman primates, close to the 3000 held by the NIH intramural program (Southwick, 1975).

T. Butler (personal communication, 1990) related that the use of nonhuman primates helped pave the way for manned space flight. In May 1952, two rhesus monkeys, Patricia and Michael, rode an Aerobee missile from Holloman Air Force Base to 36 miles above the desert and returned to earth alive. Michael Wendt, the first veterinarian assigned to the Aeromedical Laboratory, prepared the monkeys for the flight and recovered them afterward. He was among the first to serve in the largely uncharted area of nonhuman primate medicine.

8. Activities Abroad

During the 1960–1980 period, nonhuman primate centers were being developed and expanded elsewhere in the world. The Primate Center, TNO (Applied Scientific Research), was started as a local nonhuman primate resource in 1960 at Rijswijk, The Netherlands (Anonymous, 1974). In the late 1960s, TNO became a national center (Primate Center TNO, 1974). The core research program of TNO became well-known because of the work of its director, Hans Balner, an immunogeneticist. In 1974, TNO, with its 65 chimpanzees, had one of the largest captive chimpanzee breeding colonies in the world.

The most recent addition to the principal nonhuman primate centers of Europe is the German Primate Center (DPZ), completed in 1983 (Anonymous, 1983). The center is located on the university campus in Göttingen.

In Japan, efforts began in 1965 to establish the breeding of successive generations of cynomolgus monkeys (*Macaca fascicularis*). The eventual result of these efforts was the completion in 1978 of the Tsukuba Primate Center for Medical Sciences (Honjo, 1985). By 1984, the center had a colony of over 2000 nonhuman primates, with production through breeding of 200–300 monkeys. Particular research emphasis at the center has been on immunogenetics and studies of simian blood groups.

Researchers in India have used nonhuman primates for many years (K. R. Bhardwaj, personal communication, 1990). The Central Drug Research Institute in Lucknow, the National Institute of Virology in Pune, the Indian Institute of Science in Bangalore, the Institute for Research in Reproduction in Bombay, and the All India Institute of Medical Sciences in New Delhi have well-established nonhuman primate programs, as does the new National Institute of Immunology, also in New Delhi.

In Kenya, the Tigoni Primate Research Center was established in 1958 by Cynthia Booth and Louis Leakey, the world famous anthropologist (Else, 1978). In about 1980, the center was moved to a 300-acre tract and modern facilities in the Kajiado District near Nairobi. It was renamed the Institute for Primate Research (IPR) at that time. The IPR has received consistent support from the government of Kenya and through the National Museums of Kenya and its director, Richard Leakey (the son of Louis Leakey). Subcontracts from several RPRCs of the NIH and their close technical cooperation with the IPR have helped in its development. As the first director of the IPR, James Else was influential in its early growth and development as a major nonhuman primate research facility. Today, the IPR has active field and laboratory research programs in infectious diseases, reproductive biology, and breeding.

B. Constraints and Learning to Live with Them

1. Conservation Becoming a Concern

Growing realization that the resources of the world are finite and that steps had to be taken to assure the survival of many plant and animal species led to the enactment of the United States Endangered Species Conservation Act of 1969. The Endangered Species Act of 1973 extended the previous legislation

and established a threatened category of wildlife. A permit system limited the importation of listed species to scientific research, species propagation, and survival purposes. A number of nonhuman primate species were listed as endangered and 12 species were designated as threatened. However, the act did not pose a significant problem for the importation of major species used in biomedical research with the exception of the cotton-topped tamarin (*Saguinus oedipus*) and gibbons (*Hylobates* spp.).

The supply of wild-caught nonhuman primates was seriously affected by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). The United States was the first country to ratify the convention, which came into force in 1975. The feature that caused alarm for the research community was that CITES member states chose to list all nonhuman primate species as either Appendix I (endangered) or Appendix II (threatened) species. The presumption was that if there was insufficient information about the status of a species, it should be listed. The provisions of CITES extended to even the most commonly used species. Permits issued by the exporting country thus became a minimal requirement for importing any nonhuman primate species.

Thse steps would not have posed serious constraints in themselves if it had not been for a rising consciousness about conservation in major exporting countries. India placed a quota of 30,000 on rhesus monkey exports in 1973 and reduced this number to 20,000 a year later (Mack and Eudey, 1984). Indian Prime Minister Morarji Desai banned the export of nonhuman primates altogether in 1978.

Other countries also took similar restrictive actions. Information provided by Kavanaugh and Bennett (1984) showed that about 95% of the monkeys imported into the United States during the period of 1964 to 1980 came from 13 countries. By 1980, all three of the major exporters, Peru, India, and Colombia, had banned exports. The others, with the exception of the Philippines and Indonesia, also had taken restrictive actions. By 1980, it had become virtually impossible to purchase a wild-caught rhesus monkey.

2. Heeding the Warnings

a. RISE OF DOMESTIC BREEDING. An increasing number of people began to express concern about the future availability of nonhuman primates in the late 1960s and to advocate increased captive breeding (van Bekkum and Balner, 1969; World Health Organization Scientific Group, 1971; Schmidt, 1969; Neurauter and Goodwin, 1972; Hobbs, 1972; Honjo and Nomura, 1972).

Serious efforts were initiated at a few sites to breed more nonhuman primates in captivity. Thorington identified 125 breeding colonies in the United States with a total of 10,293 breeders that produced 2271 live births in 1970 (Thorington, 1971). Litton Bionetics, Inc., a commercial firm in the suburban Washington D.C. area, was described as one of the largest nonhuman primate centers in the world (Goodwin, 1975a), producing about 4000 rhesus and African green monkeys and baboons for use in cancer and other research over a 10-year period beginning in 1961 (Goodwin, 1975b).

Henry Foster looked into the future in the early 1970s and saw an opportunity. Foster, the president of Charles River Breeding Laboratories, thought the time was right for doing with monkeys what he did so successfully by producing specific pathogen-free (SPF) rodents. With help from an NIH contract, Foster stocked a mangrove island in the Florida Keys, Key Losi, with 600 tuberculosis and herpesvirus B-free rhesus monkey breeders by mid-1974 (Southwick, 1975; Pucak *et al.*, 1982). The United States Food and Drug Administration (FDA) awarded an additional contract to Charles River Breeding Laboratories in 1977 to produce monkeys for vaccine testing. This was done by releasing founder stock from Key Lois on a nearby island, Raccoon Key. This has proven to be a very efficient operation and is currently successfully marketing high-quality rhesus monkeys for use in research and testing.

During this time the United States government, specifically the NIH and the FDA, also began to take steps to avoid what was increasingly being recognized as an inevitable problem in the supply of nonhuman primates. Beginning in 1973, NIH and FDA were able to allocate funds to establish specifically breeding resources. Goodwin (1975b) reviewed the status of what had been accomplished. The FDA had awarded contracts to the CPRC in Puerto Rico and the Delta RPRC to develop semifree-ranging island and corral colonies. The DRR of the NIH negotiated contract awards for two harem-type projects at Hazleton Laboratories' facility in Alice, Texas, and at Litton Bionetic's facility in Yemassee, South Carolina. DRR also awarded a contract to breed squirrel monkeys at the CPRC. At the same time, the intramural Division of Research Services (DRS) of the NIH was making contract awards for a rhesus monkey harem-type breeding projects at Perrine, Florida, with the Papanicoulou Institute and at Gulf South Research Institute's facility in New Iberia, Louisiana.

By 1975, more than 4000 rhesus monkeys were committed to breeding at 14 major sites (Southwick, 1975). Additional breeding stocks, including other species of macaques, baboons, patas monkeys, chimpanzees, squirrel monkeys, and callitrichids, in decreasing order, brought the total number of nonhuman primate breeders in the United States in 1975 to about 5800.

Some 5000 rhesus monkeys were produced each year from domestic breeding programs by 1984 (Johnsen and Whitehair, 1986). The same report shows that at least 25 other species were also being bred in significant numbers, bringing the total to about 8000 nonhuman primates being produced annually.

b. INTERAGENCY PRIMATE STEERING COMMITTEE (IPSC). The United States Assistant Secretary for Health established the

IPSC in 1975 to provide a coordination and operational point between concerned federal agencies to assure that critical United States research and testing needs for nonhuman primates were met (Anonymous, 1975; B. D. Blood, personal communication, 1990). The IPSC was located within NIH and was chaired by Joe Held. Benjamin Blood, a veterinary public health specialist with an extensive background in international health, was selected as the IPSC executive director. The IPSC quickly moved to promote a number of projects in several countries of Latin America and Asia. One helped to establish the Primate Reproduction and Conservation Station in Iquitos, Peru, in 1976. The station included facilities for captive breeding as well as a field component for trapping and monitoring. This was probably the first serious effort in sustained yield cropping of forest populations of nonhuman primates.

One of the most important accomplishments of the IPSC was to develop the National Primate Plan (Held, 1978). Another was to lay the groundwork, through a series of sponsored meetings and reports, for the National Chimpanzee Management Plan (NCMP).

c. Other Conservation Activities. The proceedings of a major international conference on nonhuman primate conservation in 1985 indicated that the research community was taking nonhuman primate conservation and breeding seriously (Benirschke, 1986). At least half of the papers presented were from field and laboratory investigators and activities supported by biomedical and health research sponsors.

The Washington RPRC supported original studies and surveys of nonhuman primate populations in Indonesia (Smith, 1975). This center also operated a field station on the east coast of Kalimantan. Wisconsin RPRC staff member Stephen Gartlan conducted nonhuman primate field studies in Cameroon for many years and worked to establish national wildlife reserves in that country. The Animal Resources Program in NIH's DRR also provided grant support for many years to field investigators such as Charles Southwick. Southwick's longitudinal studies of Indian macaques helped to document the case for improved conservation practices and captive breeding (Southwick and Lindburg, 1986).

Approaches to improved conservation also found application closer to home. The Washington RPRC, with NIH contract support, launched the Primate Information Clearinghouse, with its "Clearinghouse Bulletin," in 1977. Its purpose was to assist in the recycling of nonhuman primates and tissues between institutions.

3. Organizations

A number of organizations and activities in the private sector played a role in the development of medical primatology. Several predated the 1970s and they did much to influence developments in primatology during this time.

The International Union for the Conservation of Nature (IUCN) was established in 1948 (S. Edwards, personal communication, 1990). The "Red Data Book" of the IUCN, containing listings of the survival status of many plant and animal species, has been used as a primary reference source for organizations such as CITES and national regulatory authorities. The Primate Specialist Group of the IUCN helped develop a "Policy Statement on Use of Primates for Biomedical Purposes" that was adopted by WHO in 1982. The statement contains recommendations that include significant limitations on trapping wild nonhuman primates and limiting their use to the establishment of captive breeding projects.

The establishment of The Animal Care Panel in 1950 [later to become the American Association for Laboratory Animal Science (AALAS)], the Institute of Laboratory Animal Resources (ILAR) in 1953, and the American College of Laboratory Animal Medicine (ACLAM) in 1957 has been well documented (Cohen and Loew, 1984). The publications of the AALAS have provided a major outlet for dissemination of information on nonhuman primate husbandry and medicine. In its technical information and standard setting role, ILAR has published materials of fundamental importance to the field. Many veterinary specialists in laboratory animal medicine certified by ACLAM have been active in the field of nonhuman primate medicine as have the veterinary members of the American Society of Laboratory Animal Practitioners which was established in 1966.

A workshop for primate veterinarians was initially organized in 1973. It formally evolved into the Association of Primate Veterinarians (APV) in 1979. The APV provided veterinarians with a forum for informally sharing information on nonhuman primate medicine and for being collectively heard on nonhuman primate matters. In the field of laboratory animals, this represented the first move toward subspecialization and was indicative of how far nonhuman primate medicine had progressed in little more than 10 years.

The International Primatological Society (IPS) and the American Society of Primatologists (ASP) began as multidisciplinary groups interested in advancing and sharing knowledge in the field of primatology. The perceived need for sharing primatological information internationally led to the establishment of the IPS in 1962, its sponsorship of the biennial International Congress on Primatology, and publication of the *International Journal of Primatology* beginning in 1979 (Anonymous, 1978). The ASP began to sponsor national meetings in the United States in 1976 and its activities have broadened to include areas such as support for nonhuman primate conservation. The ASP began publishing the *American Journal of Primatology* in the early 1980s. The first issue of the other major United States publication dedicated exclusively to nonhuman primates, the *Journal of Medical Primatology*, appeared in 1982.

The International Primate Protection League (IPPL) began to be noticed in the early 1970s. Its chairperson, Shirley

McGreal, drew public attention to the dark side of nonhuman primate supply and research through the periodic newsletter of the IPPL.

C. Transition to the 1980s

1. Patterns of Usage

Levels of "usage" of nonhuman primates during the 1970s and 1980s remained relatively constant. Usage has generally ranged from 50,000 to 60,000 animals through 1987 (Hackerman, 1988). Domestic breeding programs accounted for the supply of an increasing number of animals. In 1984, domestic supply and imports totaled 22,591 (Wolfle, 1983). Reported national usage for that year was over 57,000 (Anonymous, 1985). Reliable statistics continued to be difficult to obtain. However, it is clear that "recycling" was becoming more common and that many animals were being assigned to two or more consecutive projects each year or were being assigned to more than one project at the same time.

Chimpanzees probably provided the best example. Beginning in 1975 with the advent of CITES, importation of chimpanzees into the United States ceased for all practical purposes. Their cost also soared. Many chimpanzees were typically used in a succession of experiments in an NIH, FDA, and Centers for Disease Control (CDC) consortium established to conduct hepatitis research. Many of these chimpanzees, because they were chronically infected and shedders of hepatitis B virus, were considered unsuitable for breeding or other research purposes. By 1980, research usage was not a limiting factor in the longevity of chimpanzees. Few animals died, or were allowed to die, of research-associated mortality. The provision of appropriate housing and care for older chimpanzees that had outlived their usefulness in research began to become a serious problem.

2. Retroviral Disease

Among the vast amount of knowledge about nonhuman primates that was accumulated during this period was a development, little noticed at the time, that would significantly affect the future of nonhuman primatology. In 1967 an outbreak of lymphoid disease, including lymphomas, was reported in baboons at the IEPT in Sukhumi (Rabin, 1985). The disease appeared to be transmissible and involved immunodeficiency. During the period of 1969 to 1972, a number of gibbons (*Hylobates lar*) at the United States Army's Component of the SEATO Medical Research Laboratory in Bangkok died from a similar disease (De Paoli *et al.*, 1973). From 1969 through 1975, there were 43 cases of spontaneous lymphoma in macaques (*Macaca mulatta* and *Macaca arctoides*) at the California RPRC (Henrickson *et al.*, 1983). Tumors in nonhuman primates up to that time had been considered rare.

IV. 1980s AND 1990s: PROGRESS PAYING OFF IN FACE OF SERIOUS CHALLENGES

A. AIDS: Acquired Immunodeficiency Syndrome

1. AIDS Research and Regional Primate Research Centers

In 1984, type D retroviruses were implicated as a cause of the naturally occurring disease that had been seen earlier at the California RPRC as well as at several other centers (Letvin *et al.*, 1983; Daniel *et al.*, 1984; Marx *et al.*, 1984). Very soon afterward, simian T cell lymphotropic virus III [later renamed simian immunodeficiency virus (SIV)] was discovered at the New England RPRC (Daniel *et al.*, 1985). The SIV was given experimentally to macaques and caused an immunodeficiency disease that closely resembled human AIDS. It provided an excellent model system for research on prototype vaccines and drugs to combat AIDS. For example, later work at the New England RPRC led to the successful testing of a deletion mutant live attenuated SIV vaccine and opened a very promising approach to development of similar live vaccine to protect against HIV (human immunodeficiency virus) infection and AIDS (Daniel *et al.*, 1992). The regional nonhuman primate research centers had clearly become front line players in the rapidly expanding national AIDS research effort.

Acquired immunodeficiency syndrome provided a powerful reason for justifying earlier investments in nonhuman primate research and resources. Without this investment, and the foundation of work that was done on viral oncology and retroviruses, AIDS research could have been delayed for many years. Funds provided to the centers for AIDS research were also used to expand breeding and provide special containment facilities that were needed. This occurred at a time when funding for other areas of research in general was level or declining.

B. Emergence and Impact of Animal Rights Movement

Nonhuman primates had not been of great interest to antivivisectionists or animal protectionists prior to the 1980s. The first of two developments that firmly placed nonhuman primates on the front line of public concern occurred in July 1980. Alex Pacheco, founder of the People for the Ethical Treatment of Animals (PETA), made an animal cruelty complaint to local police about conditions and practices in the Institute for Behavioral Research laboratory of Edward Taub in a Washington, D.C., suburban area. Pacheco had signed on as a worker in the laboratory and used the opportunity to assemble documentation, including pictures (McCabe, 1990), of the nerve regeneration studies in macaques being done there. All of Taub's monkeys were confiscated by the police and placed by the court in the custody of NIH.

The charges against Taub were dismissed at an early stage but controversy continued about whether researchers or animal

rights activists would eventually gain custody of the animals. The issue was contested through the remainder of the decade and provided a good stage to keep it in the public eye. "Free the Silver Spring Monkeys" became a rallying cry that helped put PETA on the map and helped to establish animal rights as a growth industry.

The second major success of PETA was achieved as a result of the Animal Liberation Front (ALF) raid on the University of Pennsylvania's Head Injury Laboratory in 1984. Using videotapes stolen in the raid, PETA assembled a 30-min film documenting trauma and mistreatment of baboons. The film, "Unnecessary Fuss," drew its title from comments about PETA's disclosures by a senior NIH official. The film attracted the attention of the public and particularly a host of Congressional representatives who felt that the status quo was not adequate to assure the welfare of animals used in research.

Congress passed amendments to the Animal Welfare Act in late 1985 which sought to address the perceived shortcomings. Included in the various provisions were positive ideas which enjoyed wide acceptance. These included strengthening veterinary care and requiring institutional review of animal care and use. One provision that did not fall into this category was also folded into the legislation as a result of late-hour agreements reached outside the earshot of open hearings. Senator John Melcher, a veterinarian, insisted on the inclusion of language to assure the psychological well-being of nonhuman primates used in research. Although a legitimate concern, many felt the idea represented inappropriate legislation. As the decade closed 4 years later, Melcher lost his bid for reelection and sparring continued for several years more before the idea he fostered was finally negotiated into federal regulations.

Through separate legislation, the PHS and NIH were also required to implement a strengthened Animal Welfare Policy. Following extensive negotiations spanning more than 4 years, many of which focused on the issues of housing and care standards for nonhuman primates, the PHS and USDA were able to achieve some degree of consistency between their two mandates.

Although a number of legislators probably wished that the issue of research using animals would go away, they were presented in 1986 with the results of a Congressional Office of Technology Assessment study which reaffirmed that animals would be necessary for biomedical research and testing for the foreseeable future. Faced both with this reality and the cost of improvements for animal care programs required by the legislation it had passed, Congress appropriated some funds to improve research animal facilities. Although the funds appropriated were modest compared to actual needs, a significant portion of the funds were spent to help meet the new federal standards for nonhuman primates.

Concern in the scientific community about addressing needs for environmental enrichment and assuring psychological well-being in laboratory nonhuman primates led to an ILAR study and a 1994 report on the subject (T. L. Wolfle, personal communication, 1993). The report documented how much the character of captive management of nonhuman primate populations had changed with the legislation and pointed toward the continuing changes and challenges in this area that could be expected in the future. Even its harshest scientific critics would be hard pressed to say that the animal rights movement and legislation did not bring about some fundamental improvement in the way nonhuman primates were used in research.

C. Other Effects of AIDS Research

1. Chimpanzee Breeding and Research Program

In 1984, chimpanzees were found to be susceptible to infection with HIV (Alter *et al.*, 1984), resulting in an immediate interest in using available chimpanzees to test a variety of approaches to providing protection against AIDS infection. Officials at NIH and others with concerns about the limited numbers of chimpanzees available for research in the United States, about 1300, quickly came to two conclusions. First, there were probably insufficient chimpanzees in the United States to meet the potential research needs. Second, if used for such purposes, there would soon be no suitable chimpanzees left for breeding.

Responding to these concerns, NIH Director James Wyngaarden in 1985 allocated $4.5 million for AIDS research to establish the Chimpanzee Breeding and Research Program (CBRP) (Johnsen, 1987). The program was one element of the NCMP that had been developed earlier. It probably would have gone unnoticed and unfunded if it had not been for AIDS. In addition, the Public Health Service AIDS Animal Models Committee was established at NIH. The committee was charged with overseeing and approving the assignment of chimpanzees for AIDS research projects sponsored by PHS agencies. The committee helped to slow the rush to use scarce chimpanzees.

The DRR of the NIH made five awards in 1986 to support chimpanzee breeding. These were at the Primate Foundation of Arizona in Tempe, New Mexico State University's Primate Research Institute at Holloman Air Force Base in Alamagordo, the University of Texas System's Center in Bastrop, the Gulf South Research Institute at New Iberia in Louisiana, and the Yerkes RPRC. The breeding program included many of the chimpanzees available and suitable for breeding in the United States. Plans called for the program to produce about 60 offspring each year, with about half of these to be retained for future breeding. The remainder were to be assigned on a priority basis for AIDS research, if needed.

Establishment of the CBRP was controversial. Animal rights organizations and individuals such as Jane Goodall actively campaigned against the program. They feared that the increased need for chimpanzees would threaten wild populations in Africa. They argued that chimpanzees were inappropriate models for AIDS research because none had come down with clinical disease following infection with HIV. Letters poured into NIH

through congressional representatives from constituents demanding explanations.

Curiously, opposition to the CBRP never seemed to take into account that its primary goal was to assure the survival of chimpanzees. Yielding to pressure from protectionists, the United States Department of Interior did decide in 1989, as a result of continued habitat destruction and predation in Africa, to declare the chimpanzee an endangered species in its natural habitat. However, in a bow to the success of domestic breeding and conservation, the action exempted the United States population of chimpanzees.

2. Herpesvirus B and AIDs Providing Stimulus for SPF Breeding

The most serious incident ever recorded of human infection with herpesvirus B occurred in 1985 at the Navy's Aeromedical Research Laboratory in Pensacola, Florida (Palmer, 1987). The outbreak, in which four people were infected and two animal caretakers died, provided yet another reminder of the seriousness of the herpesvirus B problem and its prevalence in rhesus monkeys. It also raised concerns about the potential activation of herpesvirus B infections in monkeys that might be infected, naturally or experimentally, with retroviruses causing immunodeficiency. Already looming as a potential problem, based on the discovery of SIV, was whether there would be enough monkeys to meet the needs of AIDS research.

Both herpesvirus B and AIDS provided good arguments for breeding more SPF monkeys. The NIH responded by providing AIDS research funds and making six awards in 1989 to establish the SPF Breeding and Research Program (M. A. April, personal communication, 1990). Awards were made to Hazleton's Texas Primate Center in Alice, the New England RPRC, the University of Texas' Center in Bastrop, Laboratory Animal Breeding Services, Inc., in Yemassee (inheritor of the Litton-Bionetics operation at Yemassee and Morgan Island, South Carolina), the University of Miami at Perrine, and the Michigan Department of Public Health. These projects are projected by 1995 to produce 500 SPF rhesus monkeys per year which are free of identifiable simian retroviruses, herpesvirus B, and Ebola (or filovirus) infection.

A parallel effort in SPF breeding was undertaken at about the same time at the Washington RPRC. In cooperation with the Oregon RPRC, the Bowman Gray School of Medicine, and the Government of Indonesia, an agreement was negotiated to establish a sustained yield, free-ranging production colony of cynomolgus macaques on Tinjil Island off the southern coast of Java. Through the Interagency Research Animal Advisory Committee, the successor of the IPSC, a number of federal agencies contributed funds to start this important project along with funds for AIDS research that were provided through the RPRC program.

The colony was intended to provide a relatively low cost resource of SPF monkeys for both Indonesian and United States investigators with priority being given in the latter case to AIDS research. Many hoped too that the project would prove to be a good example for establishing similar breeding and conservation activities elsewhere in the developing world.

D. Ebola Virus and Interruption of Imports

In 1990, the CDC and the New York State Department of Health took steps that resulted in suspending the importation of macaques from southeast Asia. These actions resulted from the discovery of Ebola (filovirus) infection in cynomolgus macaques imported from the Philippines (Dalgard *et al.*, 1989). Many commercial air carriers also stopped carrying these species (Held, 1991). Follow-up studies indicated that infections with the agent were widespread and probably had gone undetected for some time. Inapparent infections in people handling infected monkeys further suggested that the agent was not as hazardous for people as initially feared (Miller *et al.*, 1990). The incident reaffirmed once more, perhaps for the last time, that dependence on the supply of wild-caught nonhuman primates involves more risks than the research community can afford.

V. LOOKING TOWARD THE FUTURE

This perspective reveals several clear trends since the 1940s that will likely shape the future use of nonhuman primates in biomedical research and testing. More work with nonhuman primates will be done in fewer places. Those places will increasingly have the comprehensive character of nonhuman primate centers with their own breeding programs, specialized facilities and operations, and multidisciplinary staff. These researchers, clinicians, and technicians will continue to add to the progress and developments that have already occurred to improve nonhuman primate medicine and to advance knowledge in primatology. More work will be done with fewer nonhuman primates and will be limited to fewer species. With new knowledge, such work will be done with greater sophistication. Biotechnology, through developments similar to the transgenic mouse, may provide alternatives to procedures such as neurovirulence testing and such testing is likely to be better. The use of wild-caught nonhuman primates in research and testing will, of necessity, become history. The fruits of research that have historically involved gaining knowledge of value to people from nonhuman primates will increasingly find applications to benefit nonhuman primates.

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CHAPTER 2

Laws, Regulations, and Policies

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NONHUMAN PRIMATES IN BIOMEDICAL RESEARCH: BIOLOGY AND MANAGEMENT

I. INTRODUCTION

This chapter summarizes the various laws, regulations, treaties, policies, and standards applicable to the management, care, and use of nonhuman primates. Besides the numerous requirements that apply to all laboratory animals, additional directives specific for nonhuman primates due to public health and conservation concerns exist. In addition, 1985 amendments to the Animal Welfare Act require institutional programs that enhance the psychological well-being of nonhuman primates. Compliance requires a thorough knowledge and intent of these requirements coupled with an understanding of the biology and behavior of specific species of nonhuman primates, their health needs, conservation, and public health issues.

Federal laws (statutes) are organized annually into their appropriate subjects (e.g., Agriculture) and are published as the United States Code (e.g., title 42 USC 264). The United States Code is comprised of all federal statutes of a general and permanent nature, arranged by subject, and are available in legal libraries. The code includes a brief intent of congress for establishing these statutes plus interpretations from federal and state court rulings. Newly passed federal statutes are published individually as public laws with a unique notation denoting the congressional year and chronological number (e.g., PL 89-111). At the end of each calendar year they are then collated in the United States Code.

Federal regulations are published in the Code of Federal Regulations (e.g., 21 CFR 71). Federal regulations are detailed requirements developed by the respective executive department (e.g., USDA, DHHS) responsible for enforcing the corresponding statute. Congress determines the executive department to which enforcement and responsibilities are assigned. Newly written regulations are published in the Federal Register as proposed rules. Following public comment, they are then published again in the Federal Register as final regulations before being incorporated into the CFR. Enforcement of the law begins only after publication of the final regulations.

Executive orders can be issued by heads of their respective executive department. They can cite specific or general laws for their authorization of these orders. Likewise the executive departments can rescind or continue such orders. They can be challenged in court through the litigation process. For the legislative body to become involved, new laws must be passed or existing ones amended.

Litigation is a process for challenging laws, regulations, and executive orders by turning to the courts. The outcome of such judicial rulings serve our society's traditional means of current interpretation of the laws of the land. Litigation can also be used by one party to force a new interpretation, challenge a current interpretation, or even as a means to express a particular viewpoint by delaying activities. Litigation has been used by a few special interest groups as a means to express their particular ethical and political viewpoint.

Professional standards are published documents on selected topics prepared by experts in respective fields of veterinary medicine, primatology, and biomedical research. These standards are influential in advancing leading improvements in nonhuman primate care and use. These professional standards are accomplished by professional organizations setting policies, publishing standards, the peer review process, and formal accreditation recognition. Accreditation by an organization such as the American Association for Accreditation of Laboratory Animal Care (AAALAC) provides a peer review process for evaluating laboratory animal care and use, programs for appropriate application of these standards and regulations. These professional procedures continue to improve the humaneness of laboratory animal care and use, provide adequate veterinary services, and promote quality science.

II. NATIONAL LAWS, REGULATIONS, AND POLICIES

A. Background and History

In 1974, the Interagency Primate Steering Committee (IPSC) became the focal point of efforts by several United States government agencies to ensure an adequate supply of nonhuman primates for biomedical research, testing, and vaccine development. This government action was taken in anticipation of the restriction on importation and the eventual elimination of rhesus monkey availability from India and neighboring countries. The primary emphases of the IPSC were to establish breeding colonies in the United States; to encourage limitation of primate use to projects requiring them; to establish the Primate Supply Information Clearing house (University of Washington) to facilitate primate conservation in the United States through sharing of resources; and to promote conservation activities in source countries. Special attention was given to developing stable domestic sources of rhesus monkeys and chimpanzees and to ensuring predictable and reliable sources. Through a contract with the Pan American Health Organization (PAHO), the IPSC also sponsored conservation efforts in South America and worked to provide a stable supply of New World primates for research.

By 1983, there was a clear need for an interagency committee with broader membership to deal with issues involving all research animal species. This need had been highlighted when the state department asked the IPSC to represent the United States in the Council of Europe's efforts to develop a draft convention on the use and protection of animals for scientific purposes and to develop a formal United States position on the draft. The IPSC was the only federal interagency committee reviewing issues involving research animals. Therefore, in 1983 IPSC became the Interagency Research Animal Committee (IRAC) with the inclusion of all federal agencies concerned with the use

of animals in biomedical research and testing. The IRAC plays a major role in governmental policy formulation, especially in the care, use, and conservation of laboratory animal species.

The areas of responsibility and concern of the IRAC are many. One of its earliest activities was to review and comment on the draft of the "U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals" (PHS Policy: implemented in 1985). This policy proclaims on its first page that it is intended to implement and supplement the "U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training," a document developed by the IRAC. This broadly accepted set of principles is familiar to the laboratory animal care and use community, appearing as it does both in the PHS Policy and the "Guide for the Care and Use of Laboratory Animals" (Guide). The committee also consulted with the U.S. Department of Agriculture on the best means of accomplishing the regulatory objectives of the department in developing regulations to implement and enforce the 1985 amendment to the Animal Welfare Act (AWA).

The efforts of the IPSC and IRAC in rhesus monkey and chimpanzee management planning have "borne fruit" as exemplified by the creation of stable United States breeding colonies of rhesus monkeys (both governmental and commercial) and the NIH National Chimpanzee Breeding and Research Program (NCBRP), funded by the NIH. The latter program has successfully produced sufficient chimpanzees to meet foreseeable breeding and biomedical research needs, and may become one of the most important factors in chimpanzee survival as the destruction of native habitats continues. Primates remain a special interest of government agencies because of their unique value in research and the many complex issues related to their use.

B. Federal Agencies

1. Department of Interior (Fish and Wildlife Service)

The Fish and Wildlife Service (FWS) regulates the trade and transportation of nonhuman primates under its responsibilities for enforcing: (1) the Lacey Act; (2) the Endangered Species Act; and (3) the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES).

a. THE LACEY ACT. This law (PL 95-1073, amended PL 97-89; 50 CFR Part 14) regulates the transportation of wild mammals and birds imported into the United States. Implementing regulations were promulgated by the Fish and Wildlife Service and enforcement of the law became effective on February 1, 1988. In promulgating the Lacey Act regulations, the FWS integrated existing requirements of the live animal transportation guidelines of CITES, the International Air Transport Association (IATA), and those of the U.S. Animal Welfare Act (AWA). Provisions of the Lacey Act regulations, therefore, are essentially identical to those of IATA, CITES, and the AWA, although some requirements for primates differ notably in methods for providing transport cage ventilation openings. Information about the Lacey Act may be obtained from the Federal Wildlife Permit Office, U.S. Fish and Wildlife Service.

b. U.S. ENDANGERED SPECIES ACT–TITLE 16 U.S. CODE 1531-1543 (CONSERVATION) 50 CFR. The Endangered Species Act was passed on December 28, 1973 to prevent the extinction of many species of animals and plants. It places restrictions on a wide range of activities involving endangered and threatened animals to help ensure their continued survival. With limited exceptions, the law prohibits activities with these protected species unless authorized by a permit from the FWS. The prohibitions apply equally to live or dead animals, their progeny, and any parts or products derived from them. Endangered species, i.e., those in danger of extinction, may be imported only under exceptional circumstances for scientific research designed to benefit the species or to enhance the propagation or survival of the species. Threatened species are those likely to become endangered within the foreseeable future. The law also regulates export, import, "take," interstate, and foreign commerce of protected species. Special Rule [50 CFR 40(c)] provides exceptions for certain primate species imported into the United States prior to November 18, 1976, as well as their progeny born after that date. Under this special rule, a permit is not required for interstate sale or export. Species exempt under those conditions include: black howler monkey, stump-tailed macaque, Formosan rock macaque, Japanese macaque, Toque macaque, lesser slow loris, pygmy chimpanzee, chimpanzees, long-tailed langur, purple-faced langur, Tonkin snub-nosed monkey, white-footed tamarin, Philippine tarsier, and Gelada baboon.

The permit office of the FWS may issue permits for otherwise prohibited activities for the following purposes: (a) endangered species permits for scientific research; enhancement of propagation or survival of the species; and incidental "taking" and (b) threatened species permits for scientific research; enhancement of propagation or survival of the species; zoological, horticultural, or botanical exhibition; educational purposes; special purposes consistent with the intent and policy of the Endangered Species Act; and incidental taking.

i. Captive-bred wildlife. Qualified persons who register with the FWS may buy and sell live endangered or threatened animals: (1) not native to the United States, (2) that have been born in the United States, and (3) for enhancement of propagation, provided the other person is registered for the same species.

ii. Applying for a permit. Permit applications and instructions may be obtained from the FWS. An application and processing fee is required, and applicants should allow at least 60 days for processing of these applications.

iii. Permit exemptions. Pre-Act or "Grandfather" clause: Species held in captivity on December 28, 1974, are exempt

from prohibitions of the act provided such holding or use of the specimen was not a commercial activity. A commercial activity is one intended for profit or gain. An affidavit and supporting documents (evidence of pre-Act status) must accompany the shipment of the listed species.

c. ENDANGERED STATUS FOR CHIMPANZEES AND PYGMY CHIMPANZEES—FINAL RULE MARCH 12, 1990. The FWS reclassified wild populations of chimpanzees and all populations of pygmy chimpanzees from threatened to endangered. However, United States captive populations of chimpanzees will remain classified as threatened and the FWS will monitor their status annually.

d. CONVENTION ON INTERNATIONAL TRADE IN ENDANGERED SPECIES (ALSO SEE SECTION IV). The United States, as a party to the convention, delegated authority to the FWS to implement the treaty provisions and provide close continuity with the U.S. Endangered Species Act. The FWS established an Office of Management Authority and an Office of Scientific Authority.

e. OFFICE OF MANAGEMENT AUTHORITY, FWS. This office has three primary functions: (1) issuance of a permit involving protected species; (2) development of United States policy on the implementation of CITES; and (3) administration of grant and technical assistance programs to other countries for the conservation of endangered species.

f. OFFICE OF SCIENTIFIC AUTHORITY, FWS. This office reviews available data on animal numbers to assess whether or not proposed export or import will be detrimental to the species. This office is also responsible for developing United States proposals and receiving foreign proposals to alter the CITES listing by adding or deleting a species. Finally, this office provides scientific assistance, both nationally and internationally, on the implementation of the provisions of CITES.

2. Department of Health and Human Services (U.S. Public Health Service)

a. NATIONAL INSTITUTES OF HEALTH. The Public Health Service Policy on Humane Care and Use of Laboratory Animals was mandated by 1985 amendments to the Public Health Service Act (Health Research Extension Act of 1985).

Through the Health Research Extension Act, institutions receiving PHS funds for activities involving vertebrate animals are required to file an Animal Welfare Assurance (assurance) with the Office for Protection from Research Risks (OPPR). The assurance, once approved by OPRR, serves as a binding agreement between the institution and the PHS legally committing the institution to bide by the PHS policy. It provides a description of the program of animal care and use (ACU), and of the Institution's Animal Care and Use Committee (IACUC) procedures for review and approval of proposed activities. The ACU program must be consistent with the "Guide for the Care and Use of Laboratory Animals" and the Animal Welfare Act.

The ACU program description must include organizational structure and lines of authority; qualifications of the veterinarian; composition of the IACUC; employee occupational health and training programs; an animal facility description; and animal census information.

Each research facility must establish at least one IACUC appointed by the chief executive officer. The PHS policy requires that the committee must be composed of at least five members (a veterinarian, a scientist experienced with animal research, a nonscientist, a member unaffiliated with the facility, and at least one other member). The AWA does not stipulate that one of the members must be a nonscientist. The IACUC responsibilities include reviewing all proposed and ongoing activities involving animals, approving all experimental animal studies, advising senior institutional officials about research animal issues, conducting semiannual evaluations of all animal facilities and programs, and providing detailed record keeping and reporting documents.

Institutions receiving PHS monies that are accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC) are allowed to substitute their accreditation status in lieu of submitting a detailed description of their program along with their assurance letter to OPRR. A failure of the institution to comply with the terms and policy conditions of their assurance may result in sanctions including the termination of PHS fiscal support for all projects involving animals.

Under the PHS policy, the training of scientists, animal technicians, and other personnel involved with animal care must be provided by the institution. The training program should include (1) humane techniques of animal care and use; and (2) techniques that minimize "the number of animals required to obtain valid results and minimize animal distress."

b. NATIONAL CHIMPANZEE MANAGEMENT PLAN (NCMP). In 1976 the United States became a signatory to CITES. As such, zoos and research institutions could no longer import chimpanzees from Africa. To meet the continuing need for chimpanzees in biomedical research and testing, cooperative agreement awards were made to five private and university-owned primate holding facilities for the specific purpose of establishing a national chimpanzee breeding program. The NCMP was endorsed by IRAC in 1984. It calls for a "self-sustaining colony capable of providing a stable supply of animals for future research." The essential elements of the NCMP include (1) a healthy and disease-free defined breeding colony; (2) that selected offspring be maintained in the colony as designated future breeders; (3) that these future breeders be maintained in a manner conducive to the learning of reproductive and parenteral skills; and (4) an established committee with national expertise to review the management and operation of this program.

c. INTERAGENCY ANIMAL MODEL COMMITTEE FOR REVIEW OF RESEARCH PROTOCOLS UTILIZING CHIMPANZEES. Similar

to the IRAC, the Interagency Animal Model Committee (IAMC) grew out of the need to balance the urgent requirements for biomedical research and the conservation of chimpanzees. In 1986, the NIH formed the AIDS Animal Model Committee to review experimental protocols for the use of domestically bred chimpanzees in the AIDS research effort. The goal of the committee was to conserve captive-bred chimpanzees and, therefore, to review all studies related to the use of chimpanzees for AIDS vaccine development and efficacy testing. The membership of this committee (now named the Interagency Animal Model Committee) has been expanded to include other government agencies and its scope now includes the evaluation of all government research on chimpanzees and all uses of those animals derived from government-sponsored breeding programs. Approval by the IAMC is required for all proposed activities involving chimpanzees.

d. NIH Intramural Nonhuman Primate Management Plan. In March 1987, the NIH director requested "state-of-the-art" housing for the intramural primate population. A steering committee was formed that would coordinate the development of a plan to meet this end. The membership of this committee included scientists, veterinarians, administrators, and architects. The resulting document, the NIH Nonhuman Primate Intramural Management Plan (the Primate Plan), presents a summation of the current literature in the field of environmental enrichment and a flexible approach to designing and implementing an enrichment program in an animal facility.

e. SPF Rhesus and Cynomolgus Breeding Program. The SPF Breeding Program, established in 1988, is designed to create self-sustaining rhesus monkey breeding populations that are free from simian retroviruses (SIV, STLV, SRV) and herpes B virus. Additional objectives of the program are the development of better diagnostic tests for these viruses, improved standards for housing and husbandry, and new techniques for genetic monitoring. Five breeding facilities participate in this program.

3. Centers for Disease Control

a. Importation of Nonhuman Primates–Title 42 U.S. Code 264 (Public Health and Welfare) 42 CFR 71. The responsibilities of the PHS for preventing the introduction of communicable diseases into the United States have been assigned to the Centers for Disease Control (CDC). The regulations of the CDC on foreign quarantine contain a section on nonhuman primates (71.53) which limits their importation for scientific, educational, or exhibition purposes and expressly excludes importation for use as pets. Importers, who must be registered with the director of the CDC, must document the intended use and certify that the primates will not be transferred to other persons without proof of intended legal use. They may not ship primates to an unregistered organization until a minimum of 31 days of quarantine has been achieved. Detailed record keeping is required. An importer's registration can be revoked for violations of CDC requirements.

Importers must report to the CDC, within 24 hr, any primate disease suspected of being yellow fever, monkeypox, or Marburg/Ebola disease (filovirus). The same reporting requirement applies to any illness in a staff member that may have been acquired from a nonhuman primate. If the CDC has evidence that nonhuman primates have been exposed to a communicable disease that may endanger the public health, it may require their examination, treatment, detention, isolation, seizure, or destruction at the owner's expense.

Primates that arrive in the United States but whose importation is precluded by provisions of the CDC regulations are disposed of, at the owner's expense, under arrangements approved by CDC. Under some circumstances, the disposal could be donation to a scientific, educational, or exhibition facility. The CDC regulations on importation of primates cite other federal regulations such as the USDA Animal Welfare regulations for husbandry and care standards.

b. Filovirus Requirements of CDC. The CDC placed increased restrictions on the importation of rhesus, cynomolgus, and African green monkeys due to the outbreaks of Ebola-like virus infections in several U.S. importers' facilities during late 1989 and early 1990. Epizootics were identified in several shipments of cynomolgus monkeys from the Philippines. Rhesus and African green monkeys, even in closed colonies, were found to have antibodies to the Ebola-like virus. Because of the high human mortality experienced during Ebola and Marburg virus infections in the past, CDC took a conservative approach—imposing more stringent primate quarantine requirements (March 1990) and requiring special one-time permits to import these three species and to release them from quarantine (April 18, 1990) (see Section II,B,8).

4. Food and Drug Administration

a. Good Laboratory Practices. Title 21 U.S. Code 371, entitled "Food and Drug," and Title 42 U.S. Code 216, 262, 263 (b-n), entitled "Public Health and Welfare," authorize regulations relating to food, drugs, welfare, cosmetics, and biological products. On June 20, 1979, the FDA finalized the Good Laboratory Practice (GLP) regulations 21 CFR, Part 58. These regulations affect both federal and nonfederal facilities conducting nonclinical laboratory investigations and have the objective of improving the quality of research studies submitted to the agency. Compliance with the AWA and the recommendations of the *Guide* are required.

b. Standards for Viral Vaccines. Title 42 U.S. Code 262, entitled "Public Health and Welfare," is the law that authorizes the FDA to license and regulate the manufacture of viral vaccines and other biological products.

The implementing regulations for this law, 21 CFR 620, detail the use of certain species of nonhuman primates for the

production and safety testing of inactivated as well as live poliomyelitis vaccine and measles, rubella, and mumps vaccines. Rhesus monkeys are routinely used for neurovirulence testing of modified live poliovirus vaccine, although the regulations only specify the use of *Macaca* spp. Species permissible as sources of kidney tissue culture are *Macaca* or *Cercopithecus* spp. The cynomolgus or rhesus monkey must be used for kidney tissue cultures in adenovirus vaccine testing. For measles, rubella, and mumps vaccines, the regulation requires the use of *Macaca* or *Ceropithecus* spp. for neurovirulence testing. The FDA Commissioner has the authority to select other primate species for these tests if they are equally suitable and meet all necessary requirements for testing.

5. United States Department of Agriculture (Animal and Plant Health Inspection Service)

a. ANIMAL WELFARE ACT–7 U.S. CODE 2131-2157 (AGRICULTURE) 9 CFR PARTS 1, 2, AND 3. Part 1 (definitions) and part 2 (regulations) of the USDA Animal Welfare Act regulations were published in the Federal Register (9 CFR 54, 36112, 35183) and became effective October 30, 1989. Although Parts 1 and 2 were significantly rewritten, the major provisions remain unchanged. The language used in the final version is very similar to that in PHS policy.

Part 3 (standards) of the USDA's animal welfare regulations requires the provision of an environmental enhancement adequate to promote the psychological well-being of primates. Key aspects that institutions must address are: (1) social grouping, (2) environmental enrichment, (3) use of restraint devices, and (4) special considerations. Exemptions can be granted for the following criteria: (1) The attending veterinarian determines that following the plan could adversely affect the clinical care of primates under medical treatment; this health-related exemption may remain in effect for a maximum of 30 days and then must be reviewed again by the attending veterinarian. (2)The principal investigator justifies, for scientific reasons, that the environmental enhancement program would interfere with the objectives of the study. The IACUC must approve these exceptions and review them at appropriate intervals, but at least annually.

The 1985 amendment to the Animal Welfare Act mandates that institutions provide a "physical environment adequate to promote the psychological well-being of primates." The results of this amendment included: (1) a number of national meetings by scientists, veterinarians, and regulators, (2) a lack of consensus for a definition of the term "psychological well-being," (3) a flurry of research on means of providing "environmental enrichment," (4) publication of proposed regulations (Part 3) by the USDA on March 15, 1989, with a resulting 10,686 comments by the public. Publication of the final rules of Part 3 were published on February 15, 1991. Litigation filed by Friends of Animals led to a ruling by the D.C. District Court (Judge Richey) to have the regulations of Part 3 rewritten. The 1991 regulations continue until decisions on appeals and revised regulations are written. (5) An active role by the OMB, calling for a cost/benefit analysis of the proposed regulations, and more recently calling for greater reliance on performance standards instead of engineering standards; and (6) the formulation of the NIH Nonhuman Primate Intramural Management Plan which delineates means of achieving an enrichment program, now available through the National Agricultural Library.

Specific requirements of the USDA AWA regulations include: (1) that "animal care, treatment, and practices and experimental procedures . . . ensure that animal pain and distress are minimized . . . ," (2) that the principal investigators consider alternatives to painful or distressing procedures, (3) that certain conditions are met in any practice which could cause pain to animals, (4) that animals not be used in more than one major survival operative experiment except in cases of scientific necessity, (5) that exceptions to standards be made only when specified by research protocol, (6) that research facilities report annually to the secretary that professionally acceptable standards governing the care, treatment, and use of animals are being followed, and (7) that research facilities provide to the secretary: (a) information on painful or distressing procedures and assurances that alternative procedures have been considered, (b) assurances that unnecessary duplications of previous animal studies are avoided, (c) assurances that the facility is abiding by the aforementioned standards, and (d) an explanation for any deviation from these standards.

b. NATIONAL AGRICULTURAL LIBRARY–ANIMAL WELFARE INFORMATION CENTER. The National Agricultural Library's Animal Welfare Information Center serves as a resource on methods of animal use which minimize pain or distress and for information on reducing or replacing animal use. The center provides numerous reference articles and publications. In addition, their staff conducts literature searches for investigators relating to their specific project to identify similar published studies or alternatives to animal use.

6. Department of Transportation

The Airline Deregulation Act of 1978 and the elimination of the Civil Aeronautics Board allow increased competitiveness among airlines and broader self-authority on the selection of goods that they transport as well as setting tariffs. Airlines have refused shipment of primates due to the increased services and requirements that must be followed. Because of the Ebola-like disease outbreaks, these problems have become even more severe for managers of nonhuman primate facilities, with international carriers and air carriers based in the United States refusing to transport nonhuman primates.

7. Justice Department

The Drug Enforcement Agency, established under Title 21 U.S. Code Chapter 13, monitors the use of dangerous and nar-

cotic drugs. Institutions using nonhuman primates frequently employ controlled substances for clinical care as well as for research purposes. The record keeping and imposed fines for noncompliance of controlled substances to the institutions can be substantial.

8. State Laws, Regulations, and Policies

a. FILOVIRUS REQUIREMENTS OF THE STATE OF NEW YORK. On March 21, 1990, due to the outbreak of an Ebola-like disease in cynomolgus monkeys described earlier (see Section II,B,3,b), the commissioner of the State of New York Department of Health imposed new requirements on importers of nonhuman primates. Before cynomolgus, rhesus, or African green monkeys can be imported into or through New York state, the animals must have been quarantined for at least 60 days outside the United States and be serologically negative for filovirus antibodies at the end of that quarantine period. Once in the United States, they are to be quarantined an additional 60 days and be serologically negative at the end of the second quarantine. All serologically positive animals are to be killed, with or without active disease. Before New York implemented these strict restrictions over 80% of all imported primates entered the United States through J.F. Kennedy airport in New York. Now there is no importation of primates into New York state since primate importers have moved their operations to other U.S. international airports.

b. FILOVIRUS REQUIREMENTS OF THE STATE OF TEXAS. On April 16, 1990, due to the Ebola-like disease outbreaks, the Department of Health of the State of Texas placed a temporary embargo on the importation of cynomolgus monkeys. This was rescinded within a few months.

c. STATE OF CALIFORNIA; CALIFORNIA IMPORTATION REQUIREMENTS. California is the only state that has specific regulations dealing with importation requirements of nonhuman primates. Permits must be obtained from the California Department of Health Services, Veterinary Public Health Unit, prior to importation of any nonhuman primate. A 30-day quarantine period in state-approved facilities is required. Compliance with state guidelines for facility construction, infection control, and infections waste procedures is also required. In addition, all nonhuman primates entering California from foreign locations must be quarantined in CDC-registered facilities.

d. OTHER ORGANIZATIONS: American Association for Accreditation of Laboratory Animal Care. This is an independent nonprofit organization with the goal of improving research through quality care of laboratory animals. PHS policy (see Section II,B,2,a) encourages program managers to seek this accreditation in order to demonstrate compliance with PHS policy, the NIH "Guide," and appropriate federal regulations. For institutions that meet GLP requirements, having AAALAC accreditation significantly enhances the assurance of meeting the recommendation as published in the "Guide."

III. INTERNATIONAL LAWS AFFECTING ACQUISITION OR USE OF NONHUMAN PRIMATES

This section summarizes international laws that affect the acquisition or use of nonhuman primates in the United States, as well as major foreign national laws that potentially affect the importation of animals or biological products from the United States. As these laws are subject to change with little or no notice, it is prudent for United States shippers to seek current information about them prior to shipment. Additional information can be obtained from the addresses listed in the Appendix at the end of this chapter.

A. Convention on International Trade in Endangered Species of Wild Fauna and Flora

The most frequently cited international law affecting nonhuman primates is CITES. This convention, a treaty among over 80 signatory nations, was signed by the United States in 1973. Amendment of the U.S. Endangered Species Act on July 1, 1975 (administered by the U.S. Fish and Wildlife Service) provided the authority, which has been enforced since 1977. CITES utilizes appendices to list animal and plant species that are vulnerable by virtue of their international trade. Habitat destruction or other threats to survival not associated with international trade are not causes for listing species in CITES. This is in contrast to species declared "threatened" or "endangered" under the U.S. Endangered Species Act, which considers threats to populations from any cause.

The U.S. Scientific Authority, an autonomous committee of representatives of federal agencies, serves as the scientific authority for implementing the convention. The Management Authority, administered by the Permit Office of the Fish and Wildlife Service, performs the administrative and management functions of the convention. CITES is closely related to the Endangered Species Act, which has somewhat different requirements for importation and exportation.

CITES categorizes nonhuman primates as either Appendix I (extremely restricted) or Appendix II (less restricted). Species listed in CITES Appendix I require an export permit, signed by a legal authority of the exporting country stating that the removal of the animals will not further threaten the survival of the wild population. An import permit, issued by the U.S. Fish and Wildlife Service, Federal Wildlife Permit Office, Department of Interior, is also needed for CITES Appendix I species. In order to qualify for this permit the animals must be imported for bona fide research, education, or public exhibit purposes and contribute to the conservation of the species. Chimpanzees,

gorillas, gibbons, orangutans, lemurs, drills, mandrills, and cotton-top tamarins are among the more common primates listed in CITES Appendix I.

The importation of CITES Appendix II species requires only an export permit signed by a legal authority in the exporting country. CITES Appendix II species, with a valid export permit, may be imported without restriction, provided they are not also listed "threatened" or "endangered" under the U.S. Endangered Species Act. Exportation of CITES Appendix I and II species have requirements similar to importation. Through the International Air Transport Association (IATA) Live Animal Regulations (LAR), CITES also addresses specific details of transportation including cage size and construction, number of animals in each primary container, temperature, humidity, ventilation, feeding, and watering.

According to the Office of Technology Assessment (OTA) report "Alternatives to Animal Use in Research, Testing, and Education" (1986a), the importance of the convention to research is twofold. First, it has limited trade in nonhuman primates and a few other species favored at one time or another in experiments (Caufield, 1984). Second, continued review of the convention by signatories has served as a forum for the discussion of protection of species used in research.

Information on the importation, exportation, or reexportation of "threatened," "endangered," CITES Appendix I, or CITES Appendix II species may be obtained from the Director of the U.S. Fish and Wildlife Service. Lists of animals on CITES may be obtained from the Federal Wildlife Permit Office, U.S. Fish and Wildlife Service. Health permits, required for importation and interstate shipment, are administered by the U.S. Department of Agriculture, Animal and Plant Health Inspection Service (APHIS). In Canada, CITES permits are administered through CITES Administrator of the Canadian Wildlife Service, Environment Canada. Health permits for importation are administered through Agriculture Canada (see CITES Appendix I).

B. European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes

This convention is one of several developed by an Ad Hoc Committee of Experts for the Protection of Animals (CAHPA) of the Council of Europe (COE) and implemented in signatory and ratifying European countries through enactment of special laws. [Other conventions relevant to this text, but not exclusively to primates, include the European Convention for the Protection of Animals during International Transport (1968) ISBN 92-871-0112-4, and an Additional Protocol to the European Convention for the Protection of Animals during international transport (1979) ISBN 92-871-0147-7.] The COE is an organization of 21 European countries that convenes under numerous forums to develop "conventions" which serve as guidance for the formulation of national guidelines and laws. The convention provides that:

> Any animal used or intended for use in a procedure shall be provided with accommodation, an environment, at least a minimum of freedom of movement, food, water and care, appropriate to its health and well-being. Any restriction on the extent to which an animal can satisfy its physiological and ethological needs shall be limited as far as practicable. In the implementation of this provision, regard should be paid to the guidelines for accommodation and care of animals set out in Appendix A to this convention.

Appendix A of the convention provides *recommended* cage sizes by weight of nonhuman primates, not by species. The CAHPA convention stipulates detailed standards of husbandry and transport of research animals, including the care and treatment of laboratory animals prior to, during, and following experimentation; registry of organizations using animals in experimentation; inspection, monitoring, and record keeping; and mechanisms to determine if particular experimental procedures are permissible.

When the principles of the convention are adopted by participating countries and become incorporated into national laws, the convention serves to unify individual nation's laws. (See the United Kingdom "The Animals (Scientific Procedures) Act of 1986" discussed next as an example.) With the unification of Europe progressing at a rapid pace, these conventions are serving as templates for the promulgation of quite similar national European laws and may have a profound impact on the international trade of pharmaceuticals and other products and technologies developed with the use of animals. In order to minimize the negative impact of dissimilar national laws, official observers for the United States and the International Council for Laboratory Animal Science were present at each meeting of CAHPA. Although the observers had no voting power, their advice and knowledge of United States laws and precedence were sought. Some ICLAS members did serve on CAHPA.

A notable difference with U.S. Animal Welfare Regulations is the inclusion of rats and mice in the convention. While the direct impact of this convention may be felt most by individuals or firms shipping animals or products to Europe, all users of nonhuman primates may be indirectly affected due to the increasing tendency for "continental drift" of regulations and policies from more restrictive to less restrictive countries.

Individuals desiring additional information about any of the COE conventions should contact the relevant enforcement officials in countries of interest or contact the U.S. sales agent for COE publications (see the Appendix at the end of this chapter).

C. The Animals (Scientific Procedures) Act of 1986

The Animals (Scientific Procedures) Act of 1986 applies throughout the United Kingdom with additional controls incorporated into the laws of Scotland and Northern Ireland. Because

of the close relationship between United States investigators and institutions and those of the United Kingdom, a brief understanding of the act is appropriate even though it has no direct influence on the care and use of primates in the United States. This act revises the Cruelty to Animals Act of 1896, the oldest of the national laws regulating the use of animals in scientific activities, and implements the 1986 European Convention (COE). It is a comprehensive law covering housing and environment, animal care and health, transport, special considerations for housing and care of each commonly used species, humane killing, disposal of dead animals, and cage dimensions. The Animals (Scientific Procedures) Act of 1986 is quite similar to its predecessor, but with many new provisions taken from the Royal Society/Universities Federation for Animal Welfare (RS/UFAW) "Guidelines on the Care of Laboratory Animals and their Use for Scientific Purposes": I–Housing and Care (1987). The Code of Practice for the Housing and Care of Animals Used in Scientific Procedures, promulgated under the act, addresses in the Subsection on Special Considerations (paragraphs 3.43–3.58) the needs of nonhuman primates. Paragraph 5.5 provides tables of cage dimensions for primates based on the RS/UFAW guidelines (see Table I). These sections encourage housing that is safe for personnel and animals, social housing for the animals, ample space to permit "vertical flight reactions" and sufficient to "permit the animals to stand erect, jump, and climb, and to sit on a perch without head or tail touching the cage."

The United Kingdom Act differs profoundly from the United States Animal Welfare Act. It requires investigators to be licensed and, in addition, to obtain individual certificates for each unique experiment. Both are issued by the Home Office only to investigators judged qualified. Each licensee must file an annual report to the Home Office, whose inspectors (medical or veterinary doctors) inspect the licensee's institution on a regular basis. Additionally, each institution must have a veterinarian with responsibility for the health and welfare of the animals and for certain oversight during experimental procedures. The Scientific Procedures Act is administered by The Cruelty to Animals Inspectorate in The Home Office of the Secretary of State.

D. Canadian Council on Animal Care (CCAC)

Canada differs from both Great Britain and the United States in that it has no national animal welfare laws. A report to the Department of Justice (Canada), a Law Reform Commission whose mandate is to review all federal Canadian laws, described the cruelty to animals section of the Criminal Code of Canada as being "rooted in the horse-and-buggy era of Victorian England." This lack of modern national legislation is a tribute to the success of a voluntary program of surveillance for the care and use of laboratory animals established in 1968 by the Association of Universities and Colleges of Canada (AUCC). It is administered by the Canadian Council on Animal Care (CCAC), a private, nonprofit organization funded jointly by the Medical Research Council (MRC) and the Natural Sciences and Engineering Council (NSERC). CCAC is governed by a council of scientists and laypersons appointed as representatives of approximately 15 agencies, including membership from the MRC, NSERC, and the Canadian Federation of Humane Societies (CFHS). Compliance with guidelines, established in a two-volume "Guide to the Care and Use of Experimental Animals" and a variety of policy statements such as Ethics on Animal Experimentation," is exercised by institutional animal care committees (Rowsell, 1980).

Compliance with CCAC requirements is reviewed by periodic evaluations by an assessment panel appointed from a list of almost 200 scientists with expertise in various fields. An additional appointee by the CFHS has authority similar to the other panel members, whereas a member of the CCAC secretariat assessment program serves as an ex officio member. Besides visits that are announced, because of the documentation required, unannounced visits by members of the CCAC secretariat are becoming more common. Noncompliance with CCAC requirements may result in withdrawal of all research funding to the institution. This system is well received by Canadian academic and research institutions, investigators, and funding agencies, and is condoned by the CFHS.

Several Canadian provinces have passed laws regulating the acquisition and use of research animals that focus primarily on the acquisition of dogs from municipal pounds. The Province of Ontario, under the Animals for Research Act of 1970, has requirements that include the registration of research institutions, responsibilities of the animal care committees, use of anesthetics and analgesics to alleviate pain, and the termination of any study where pain cannot be alleviated. The Ontario Act and the CCAC program complement each other, for, like all legislation, the Ontario Act can only seek minimal requirements whereas the CCAC can reach for optimal conditions (Rowsell, 1980).

Canada and the United States place the responsibility of compliance upon the institution, as opposed to the British system that places the investigator and the named veterinarian in the position of ultimate responsibility. It is not surprising that British scientists seem somewhat better informed about animal welfare issues than are their American and Canadian counterparts; however, the latter have become increasingly more informed in recent years. The different focus of responsibility may cause the parent institutions of United Kingdom investigators to be relatively less concerned about animal care, animal care technology, the physical plant, and the supporting infrastructure, and may contribute to the perception of differences in the physical plants and the personal awareness among the different countries (Loew, 1987).

TABLE I

CAGE REQUIREMENTS FOR NONHUMAN PRIMATES IN THE UNITED STATES,[a] UNITED KINGDOM,[b] AND CANADA[c]

Weight per animal (kg)	Minimum floor area per animal (m^2)			Minimum height (cm)		
	US	UFAW	Canada	US	UFAW	Canada
<0.65		0.25[d]			80[d]	
<0.65		0.8[e]			100[e]	
<1	0.15		0.15	51		51
<1.4		0.5[f]			100[f]	
<1.4		2.0[g]			150[g]	
1–3	0.28		0.3	76		76
3–10	0.4		0.4[h]	76		90[h]
<4		0.6[i]			100[i]	
<6		0.8[i]			110[i]	
>6		1.4/25[j]			150/200[j]	
5–12			0.74[k]			91[k]
10–15	0.56			81		
>15			1.39/2.8[l]			122
15–25	0.74		0.75[m]	91		120[m]
>25[n]	2.33			213		

[a]U.S. Animal Welfare Regulations (on February 15, 1994, U.S. Animal Welfare Regulations required that this space be provided to *each* animal whether housed individually or in groups).
[b]UFAW (1987).
[c]CCAC Guide (1984).
[d]1–2 Callithrix (nonbreeding).
[e]2–6 Callithrix (breeding).
[f]1–2 Saimiri (nonbreeding).
[g]8–10 Saimiri (breeding).
[h]Macaques <7 kg.
[i]Baboons and macaques.
[j]Baboons and macaques single/group.
[k]Baboons single.
[l]Baboons single/group.
[m]Macaques.
[n]Includes great apes and brachiating species.

Each country has established somewhat different guidelines for the husbandry of nonhuman primates. Table I provides a comparison of primate caging requirements in Canada, the United Kingdom, and the United States.

E. International Air Transport Association (IATA)

International air carriers of nonhuman primates adhere closely to the Live Animal Regulations (LAR) (revised at least biannually) of the International Air Transport Association (IATA). Alerts published by IATA may cause immediate changes in transport policy affecting even animals on the tarmac awaiting a flight. United States shippers should be familiar with IATA guidelines and the publications of the Animal Transportation Association. Shippers should always seek to ship by direct flights to avoid problems inherent with flight changes and offloading of live cargo.

Although the "regulations" have no force of law except when they are adopted as legislation or applied under regulations of specific countries, the influence of the IATA over the transportation industry is considerable. When a filovirus was found in a shipment of cynomolgus monkeys imported from the Philippines, IATA alerted its member carriers of possible risk to their employees. The result was an immediate halt in the transport of practically all nonhuman primates by commercial carriers in the United States. Although not all countries outside of North America ceased handling nonhuman primates at this time, many of the IATA member carriers heeded the advice of IATA and limited carriage.

The 15th edition of LAR includes 11 chapters, appendices, a glossary, bibliography, sales agents, and lists of IATA members and associate members and other airlines. Chapter 4 covers requirements for 81 containers, consisting of line drawings notated with dimensions, ventilation holes, cage openings, and waste pans. The regulations present an easily recognizable container acceptable for each species. The LAR is used by shippers, agents accepting animals, baggage handlers, and flight personnel to determine whether the container is acceptable. Although potential conflict with United States and international laws affecting the transport of nonhuman primates exists, recent revisions and enhanced dialogue with the Departments of Interior and Agriculture have narrowed the areas of differences to the point that they seem to be a reasonable and accurate method to quickly assess transport containers for the safety of animals and personnel.

IV. ORGANIZATIONS WHOSE POLICIES AFFECT INTERNATIONAL ACQUISITION AND USE OF NONHUMAN PRIMATES AND OTHER SPECIES

This section provides, in alphabetical order, an overview of recommendations and guidelines from U.S. federal agencies, industries, and professional organizations that may be of interest to managers and users of nonhuman primates. A selected list of professional and governmental organizations is provided.

A. Academy of Surgical Research

A Policy Statement: The Use of Animals in Surgical Research

> The Academy of Surgical Research recognizes the importance and continuing need for the use of laboratory animals in surgical research. Without the use of laboratory animals, most of the progress made in the treatment and alleviation of diseases of humans and animals could not have been made. It is anticipated that laboratory animals will continue to be necessary to make such progress in the foreseeable future.

The Academy recognizes that only the proper selection of animal models and surgical techniques will yield scientifically valid and significant results. The Academy also recognizes that laboratory animals must be treated with respect and be utilized in a humane manner.

Consequently, the Academy recommends that personnel performing surgery on laboratory animals be properly trained in surgery, anesthesia, and ethics. Individuals and institutions should follow all applicable laws and regulations concerning the use of animals in surgical research. It is strongly recommended that eligible institutions become accredited by the American Association for the Accreditation of Laboratory Animal Care in order to assure the public of their commitment to the highest standards of laboratory animal care.

B. American Psychological Association (APA)

The American Psychological Association (APA) "Principles for the Care and Use of Animals" (1979), written by the Committee on Animal Research and Experimentation, were adopted and published by the APA Council of Representatives in September 1979.

An investigator of animal behavior strives to advance our understanding of basic behavioral principles and to contribute to the improvement of human health and welfare. In seeking these ends, the investigator should ensure the welfare of the animals and should treat them humanely. Laws and regulations notwithstanding, the animal's immediate protection depends upon the scientist's own conscience. For this reason, the American Psychological Association has adopted the following principles to guide individuals in their use of animals in research, teaching, and practical applications. All research conducted by members of the American Psychological Association or published in its journals must conform to these principles.

1. The acquisition, care, use, and disposal of all animals shall be in compliance with current federal, state or provincial, and local laws and regulations.
2. A scientist trained in research methods and experienced in the care of laboratory animals shall closely supervise all procedures involving animals and be responsible for ensuring appropriate consideration of their comfort, health, and humane treatment.
3. Scientists shall ensure that all individuals using animals under their supervision have received explicit instruction in experimental methods and in the care, maintenance, and handling of the species being used. Responsibilities and activities of individuals shall be consistent with their respective competencies.
4. Scientists shall make every effort to minimize discomfort, illness, and pain to the animals. A procedure subjecting animals to pain, stress, or privation shall be used only when an alternative procedure is unavailable and the goal is justified by its prospective scientific, educational, or applied value. Surgical procedures shall be performed under appropriate anesthesia; techniques to avoid infection and minimize pain must be followed during and after surgery. Euthanasia shall be prompt and humane.
5. Investigators are strongly urged to consult with the Committee on Animal Research and Experimentation at any stage preparatory to or during a research project for advice about the appropriateness of research procedures or ethical issues related to experiments involving animals. Concerned individuals with any questions regarding adherence to the principles should consult with the committee.
6. Apparent violations of these principles shall be reported immediately to the facility supervisor whose signature appears below:

C. American Society of Laboratory Animal Practitioners (ASLAP)

The ASLAP is a professional society of approximately 750 veterinarians engaged or interested in laboratory animal practice and who hold current membership in the American Veterinary Medical Association (AVMA), Canadian Veterinary Medical Association, or other national veterinary medical associations recognized by the AVMA. ASLAP was founded in 1966 "to promote the dissemination of ideas, experiences, and knowledge among veterinarians engaged in laboratory animal practice; encourage research in clinical problems relating to laboratory animal practice; actively encourage and assist in the training of veterinarians in the field of laboratory animal medicine at both pre- and postdoctoral levels and to lend advice to institutions conducting laboratory animal medicine training programs; and act as a spokesperson for its members before the veterinary profession and other medical organizations or groups." ASLAP is an affiliate of the American Association for Laboratory Animal Science and represents the specialty of Laboratory Animal Medicine in the AVMA House of Delegates. *Synapse*, published four times a year, is the newsletter of ASLAP.

D. American Society of Primatologists (ASP)

The ASP is a nonprofit professional organization dedicated to educational and scientific purposes. This organization promotes and encourages the discovery and exchange of information regarding nonhuman primates, including all aspects of their anatomy, behavior, development, ecology, evolution, genetics, nutrition, physiology, reproduction, systematics, conservation, husbandry, and the use in biomedical research. The ASP sponsors annual scientific sessions and recognizes the work of scientists in primate conservation through the award of research grants. The quarterly *ASP Bulletin* contains information items and research reports.

E. American Veterinary Medical Association (AVMA)

1. 1993 Report of AVMA Panel on Euthanasia

A revision of a 1986 report, this report serves as the standard reference for euthanasia of experimental animals in the United States. This revision also provides information on the public disposal of surplus animals and on slaughter of food animals. The report uses the verb form *euthanatize* and stresses those methods and agents for which there is reliable data. The preface states the ". . . overriding commitment is to give professional guidance for relieving the pain and suffering of animals."

2. American Veterinary Medical Association Committee on Surgical Research and Teaching

With a scope somewhat similar to the euthanasia report, the AVMA published in 1993 the "Guidelines for Animal Surgery in Research and Teaching."

F. Animal Transport Association (AATA)

An organization with some similar interests to the International Air Transport Association, the Animal Transport Association (AATA) is an international educational organization. The abbreviation, AATA, is derived from the previous name of the organization, the Animal Air Transport Association. When the scope was changed to include transport by land and sea, the abbreviation remained the same. According to its literature, "AATA provides an important opportunity for individuals, businesses, organizations, and groups involved in any phase of animal transportation to become part of an international effort to find solutions to a variety of problems to the transport of animals. At the same time, members are linked to information, resources, contacts, and key developments in the field that can help them provide better services and conditions for animals in transit." It further states, "AATA takes a pro-active role, intervening in crisis situations, as appropriate, to make its positions known and to help resolve problems as they occur. AATA also keeps regulatory officials and the industry informed on an ongoing basis to help prevent such situations from occurring." AATA holds annual scientific meetings and offers membership to organizations, individuals, governmental or institutional employees, and students in all disciplines interested in animal transportation by land, sea, or air. It seeks to secure support for research in the field of animal transport through the AATA Harry Rowsell Foundation. AATA has offices in the United States and in Great Britain.

G. Association of Primate Veterinarians (APV)

The APV is a professional organization of approximately 240 veterinarians concerned with the health, care, and welfare of nonhuman primates. In 1973 a workshop was held at the National Institutes of Health, which became the first annual "monkey doctors" meeting. In 1979, bylaws were adopted and APV was born. Its objectives are: "To promote dissemination of information relating to the health, care, and welfare of nonhuman primates; to provide a mechanism by which primate veterinarians may speak collectively on matters regarding nonhuman primates; and to promote fellowship among primate veterinarians." Scientific and business meetings are held annually.

H. Council for International Organizations of Medical Sciences (CIOMS)

The CIOMS is an international nongovernmental scientific organization established jointly by the WHO and the United Nations Educational, Scientific, and Cultural Organization (UNESCO) in 1949. Through its membership, CIOMS is representative of a substantial proportion of the biomedical community. The membership of CIOMS consists of over 60 international medical organizations, representing many of the biomedical disciplines, and over 25 national members. The main objectives of CIOMS are to facilitate and promote international activities in the field of biomedical sciences, especially when the participation of several international associations and national institutions is deemed necessary; to maintain collaborative relations within the United Nations and its specialized agencies, in particular with WHO and UNESCO; and to serve the scientific interests of the international biomedical community. CIOMS is notable to the United States biomedical community through its 1985 "International Guiding Principles for Biomedical Research Involving Animals" (CIOMS, 1985). The introduction of this publication states that its purpose is not to duplicate existing national regulations or voluntary codes but to provide a conceptual framework, acceptable both to the international biomedical community and to moderate animal welfare groups, for whatever regulatory measure each country or scientific body chooses to adopt in respect of the animals used for scientific purposes. It acknowledges the "U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training" (reprinted in the back of the "Guide for the Care and Use of Laboratory Animals" (1985) and is incorporated into the Public Health Service "Policy on Humane Care and Use of Vertebrate Animals" (1986) and states that they "were to a considerable extent based upon the CIOMS guiding principles." The principles consist of four parts: (I) eleven basic principles; (II) special provisions including acquisition, transportation, housing, environmental conditions, nutrition, veterinary care, and records; (III) monitoring of the care and use of animals for experimentation; and (IV) methods not involving animals: "alternatives."

I. Federation of European Laboratory Animal Science Association (FELASA)

This organization consists of the membership of laboratory animal science associations in the United Kingdom (LASA), Scandinavia (SCANLAS), Europe (GU/SALAS), and other countries. Scientific meetings are held in various European countries every 3 years.

J. Field Guidelines for Study of Animals in the Wild

Investigators who study animals in their natural habitat have developed a series of field manuals. These four guidelines, published individually by the professional societies and compiled by the Scientists Center for Animal Welfare (Orlans, 1988), include: "Acceptable Field Methods in Mammalogy: Preliminary Guidelines Approved by the American Society of

Mammalogists" (prepared by an ad hoc committee on "Acceptable Field Methods in Mammology of the American Society of Mammologists"); "Guidelines for the Use of Wild Birds in Research" (prepared by the American Ornithologists' Union, Cooper Ornithological Society, and Wilson Ornithological Society); "Guidelines for Use of Live Amphibians and Reptiles in Field Research" (American Society of Ichthyologists and Herpetologists, The Herpetologists' League, and the Society for the Study of Amphibians and Reptiles); and "Guidelines for Use of Fishes in Field Research" (American Society of Ichthyologists and Herpetologists, American Fisheries Society, and the American Institute of Fisheries Research Biologists).

K. Guide for Care and Use of Agricultural Animals in Agricultural Research and Teaching

This report provides guidance on the care of agricultural animals used in agricultural research (Agricultural Guide, 1988). Portions of it have been adopted by the American Association for Accreditation of Laboratory Animal Care. It provides the first attempt in the United States to develop guidance for agricultural animals. A revision is being considered, but it is not known at this time whether subsequent revisions will begin to address agricultural animals used in biomedical research.

L. Institute of Laboratory Animal Resources (ILAR)

The ILAR was founded in 1952 under the auspices of the National Research Council, National Academy of Sciences, a congressionally chartered nongovernmental agency. The ILAR council is comprised of scientists who oversee and direct program activities. The ILAR assists federal agencies, universities, scientists, and others by convening committees of experts who compile reports on topics about laboratory animal care and use, including a series of reports on laboratory animal management. The ILAR maintains an information database on commercial and investigator-held colonies of unique animals, publishes a resource directory, "Animals for Research," and publishes a quarterly bulletin, "ILAR News," as a resource for institutional animal care and use committees, scientists, and veterinarians.

The best known report of the ILAR is the "Guide for the Care and Use of Animals" (1985), the standard reference for laboratory animal care on which the Public Health Service "Policy on Humane Care and Use of Laboratory Animals" (1986) and the standards of the American Association for Accreditation of Laboratory Animal Care are based. Other current reports include a laboratory animal management series on the management of common and uncommon laboratory animals: "Immunodeficient Rodents: A Guide to Their Immunobiology, Husbandry, and Use"; "Principles and Guidelines for the Use of Animals in Precollege Education"; "Infectious Diseases of Mice and Rats"; "Companion Guide to Infectious Diseases of Mice and Rats"; "Recognition and Alleviation of Pain and Distress in Laboratory Animals"; "Education and Training in the Care and Use of Laboratory Animals: A Guide for Developing Institutional Programs" (a guide for institutions to use to establish investigator training programs in laboratory animal science); and "Important Laboratory Animal Resources: Selection Criteria and Funding Mechanisms for Their Preservation." New initiatives will focus on transgenic animals, including their nomenclature and husbandry; the psychological well-being of nonhuman primates; the laboratory animal management of dogs and rodents; and occupational safety and health programs.

The Standardized Genetic Nomenclature for Mice program of the ILAR, undertaken in 1984 at the request of the International Committee on Standardized Genetic Nomenclature for Mice, serves to maintain the international list of holder codes that are used in correctly designating laboratory rodents. The staff maintains a registry of this listing and assigns investigator codes upon request. This activity serves to promote correct identification of rodents through the use of standardized nomenclature.

The ILAR responds to requests from precollege students about careers in science and information for reports, term papers, and science fair projects. It also serves as a conduit to assist students in locating a nearby mentor veterinarian or other scientist with expertise in a specific research topic.

M. International Association for the Study of Pain (IASP)

The IASP Committee for Research and Ethical Issues solicited comments on guidelines for the use of animals in studies producing experimental pain and suffering in an IASP newsletter (International Association for the Study of Pain, 1982). The following guidelines were developed and subsequently published (Guest Editorial, 1983):

1. It is essential that the intended experiments on pain in conscious animals be reviewed beforehand by scientists and laypersons. The potential benefit of such experiments to our understanding of pain mechanisms and pain therapy needs to be shown. The investigator should be aware of the ethical need for a continuing justification of his investigations.
2. If possible, the investigator should try the pain stimulus on himself (sic); this principle applies for most noninvasive stimuli causing acute pain.
3. To make possible the evaluation of the levels of pain, the investigator should give a careful assessment of the animal's deviation from normal behavior. To this end, physiological and behavioral parameters should be measured. The outcome of this assessment should be included in the manuscript.
4. In studies of acute or chronic pain in animals, measures should be taken to provide a reasonable assurance that the animal is exposed to the minimal pain necessary for the purposes of the experiment.
5. An animal presumably experiencing chronic pain should be treated for relief of pain or should be allowed to self-administer analgesic agents or procedures, as long as this will not interfere with the aim of the investigation.
6. Studies of pain in animals paralyzed with a neuromuscular blocking agent should not be performed without a general anesthetic or an appropriate surgical procedure that eliminates sensory awareness.

7. The duration of the experiment must be as short as possible and the number of animals involved kept to a minimum.

[See also "Preparation and Maintenance of Higher Mammals during Neuroscience Experiments" (NIH, 1991b).]

N. International Primatological Society (IPS)

The IPS is a professional international organization of primate biologists dedicated to the study and conservation of nonhuman primates and their natural habitats. The IPS provides grants for studies in primate biology, taxonomy, and behavior and holds scientific meetings every other year in host countries. The IPS "International Guidelines for the Acquisition, Care, and Breeding of Nonhuman Primates" (1988) contains recommendations regarding capture from the wild, international shipments, institutional policies, primate holding, animal care and health, breeding in captivity, and experimental/ethical considerations. A companion "IPS Code of Practice: Housing, Care, and Environmental Enrichment" is being developed. These and other IPS reports provide useful guidance, especially for source and Third World countries with developing nonhuman primate research and conservation programs. The reports are available from the Universities Federation for Animal Welfare (UFAW).

O. National Agricultural Library

Established by the 1985 amendment to the Animal Welfare Act, the Animal Welfare Information Center (AWIC) provides extensive information on many aspects of laboratory animal husbandry, care, and use and on issues of training and education. The AWIC provides single copies of specific bibliographic searches free of charge and is a rapidly developing resource and repository.

P. National Association for Biomedical Research (NABR) and the Foundation for Biomedical Research (FBR)

The NABR is dedicated to advocating the vital role of laboratory animals in the search to relieve suffering and save lives. Established in 1979 as a nonprofit association, the NABR represents the scientific community in national policy making which affects the use of animals in biomedical research, education, and product safety testing. Members of NABR include more than 350 institutions ranging from universities, medical and veterinary schools to teaching hospitals, voluntary health agencies, and professional societies, as well as pharmaceutical companies and other research related firms. "NABR Updates" and "Alerts" are brief, timely one-sheet bulletins published frequently to keep members informed of legislative/regulatory developments on all levels of government, as well as activities of the animal rights movement.

The FBR provides the public with facts about the continuing need to use animals in biomedical research and testing. The "FBR Newsletter" is published six times per year to inform contributors of foundation activities, federal and state legislation, biomedical advances, and the activities of the animal rights movement.

Q. National Institutes of Health (NIH)

As the primary federal funding source for studies utilizing nonhuman primates, NIH is a rich resource for information. Two of its more recent publications include: "Preparation and Maintenance of Higher Mammals during Neuroscience Experiments: Report of a National Institutes of Health Workshop" (1991b); and the "NIH Nonhuman Primate Management Plan" (1991a), a strategy for environmental enrichment for nonhuman primates. For these reports and other available materials and information contact the NIH Office of Animal Care and Use (see the Appendix at the end of this chapter).

R. National Library of Medicine (NLM)

The NLM publishes Current Bibliographies in Medicine (CBM), a part of the NLM's Literature Search Series. A CBM Series Note states, "Each bibliography covers a distinct subject area of biomedicine and is intended to fulfill a current awareness function." Citations for bibliographies are derived from MEDLINE, AVLINE, BIOETHICSLINE, CANCERLIT, CATLINE, HEALTH, POPLINE, and TOXLINE. Special bibliographies are prepared on animals, laboratory animal welfare, environmental enrichment, and other topics. The NLM has developed GRATEFUL MED for use by IBM or Macintosh personal computers to make online searching easier.

S. Pan American Health Organization (PAHO)

The PAHO is the organizational component of WHO that serves the Americas. This organization provides expertise for WHO regarding sources of New World nonhuman primates used in research and testing.

1. Technical Cooperation in the Americas

Since the early 1970s, PAHO has recognized the essential role of nonhuman primates in its overall program of technical cooperation in public health. Through its veterinary public health program, PAHO has actively collaborated with its member governments in developing and strengthening activities for the conservation, reproduction, and rational use of primates. These are based on the following rationale: (1) The conservation of wild primate populations deserves special attention in order that these animals may thrive in their natural habitat on a permanent basis; (2) natural primate populations can be managed as a renewable natural resource; (3) member governments possessing natural wild primate populations stand to benefit by periodic careful

cropping, whether from controlled capture in the wild or from local breeding colonies; and (4) nonhuman primate conservation activities should impact positively as a vehicle for community development in conservation-designated areas.

2. National Primatology Programs in the Americas

National programs have been developed in the respective countries that have important native populations of nonhuman primates. Support of primate reproduction through the establishment of breeding stations and management of the free-living status of wild populations are important components of each national program, as are health, nutritional, taxonomic, and physiological studies of animals in reproduction centers. In areas in which species are not endangered, specimens will be periodically made available as breeding stock for biomedical studies.

The specific objectives of the PAHO program of technical cooperation in nonhuman primates are to (1) develop methods for breeding primates in captivity and in their natural habitat, and to promote their conservation in the wilderness; (2) determine the present status, ecology, distribution, and population dynamics of nonhuman primates, especially those needed in biomedical research; (3) provide recommendations for the protection of primate habitats; (4) provide advice for management of natural populations and breeding techniques for perpetuating species in captivity and on islands; (5) obtain information and develop methods to stabilize or upgrade the habitat for selected species of flora and fauna in the forest; (6) collaborate with national authorities in developing nonhuman primate conservation and reproduction plans; and (7) provide community-based educational programs to enhance the standard of living of persons without the destruction of primates or their habitat.

T. Scientists Center for Animal Welfare (SCAW)

The SCAW, founded in 1979, is a nonprofit, educational organization that promotes the responsible and humane treatment of animals used in research, testing, and teaching. Proceedings of conferences are published regularly, e.g., "Well-being of Nonhuman Primates in Research" (Mench and Krulisch, 1990).

U. Society of Toxicology (SOT)

Position Paper on LD_{50} and Acute Eye and Skin Irritation Tests

The following comments on acute toxicity were developed by the Society on Toxicology (SOT) Animals in Research Committee and approved by the SOT Council (1989):

> Conduct of any form of testing of potentially hazardous materials in animals, including lethality or eye and skin irritation testing, should be undertaken only after careful consideration of the necessity for, the objectives behind, and the possible alternatives to, such testing. Acute toxicity testing to determine an approximate lethal dose provides a basis for a comparison of the relative toxicities of different materials. These data are used to classify materials for transportation and labeling, to provide information for treatment of acute intoxications, to aid in dose selection for subsequent toxicity studies, and to provide comparison data for evaluation and validation of alternative methods in toxicology. Although the classical LD_{50} test provides a general estimate of the quantity of chemical likely to cause death, much of the same information can be provided by other forms of testing in which significantly fewer number of animals are employed. Acute eye and skin irritation tests on chemical substances are conducted in order to characterize the hazards associated with ocular or dermal exposure. At present, tests in intact animals are the only means of assessing the potential hazard from such exposure other than direct testing in man. Although validated *in vitro* alternatives to eye and skin irritation tests in animals are not available currently, many tests under development show promise and may be useful as initial screening techniques. Complete validation of these alternate forms of testing for irritation may reduce the need to use whole animals. Until these procedures have been thoroughly tested and validated, the investigator will have to rely on conventional methods. In each case, however, attention should be given to the design and conduct of the study to reduce the number of animals and to minimize animal discomfort.

V. Universities Federation for Animal Welfare (UFAW)

The UFAW is a science-based animal welfare society whose goal is to improve the well-being of animals which man uses and manages. It has both commissioned and carried out research on ways of improving the welfare of nonhuman primates. It cooperated with IPS in the publication of the "International Guidelines for the Acquisition, Care, and Breeding of Nonhuman Primates" (IPS, 1988) and with the Royal Society for publication of the "Guidelines on the Care of Laboratory Animals and Their Use for Scientific Purposes. I. Housing and Care" (1987) and "Guidelines on the Care of Laboratory Animals and Their Use for Scientific Purposes. II. Pain, Analgesia, and Anaesthesia" (1989). The UFAW "Handbook on the Care and Management of Laboratory Animals" (1987) is a respected source of information on a wide variety of laboratory animal topics. Other reports, such as species supplements to the "Handbook on the Care and Management of Laboratory Animals," and "Guidelines for the Recognition and Assessment of Pain in Animals" (UFAW, 1989), are valuable additions to comparative medical libraries. The UFAW has announced plans to launch a new quarterly, peer-reviewed scientific journal, *Animal Welfare*, in January 1992 and to establish a subsidiary charity, the International Academy for Animal Welfare Science, which will act as a focal point for the collection, dissemination, and exchange of information and views relating to animal welfare on a worldwide basis.

W. World Health Organization (WHO)

Through WHO headquarters in Geneva, Switzerland, more than 150 nations exchange information and share resources for laboratory animal science training, technical information, consultative support, and other activities (OTA, 1986b). The WHO

divisions of Biologics and Veterinary Public Health have specific interest in the acquisition and use of nonhuman primates.

The availability of primates for essential biomedical use is a universal concern, a matter with important repercussions for the health services of every country. The WHO has assigned a special priority to several international health programs that utilize primates in research. These include the Expanded Program on Immunization, the Tropical Diseases Research Program, and the Special Program in Human Reproduction, all of which rely heavily on the use of nonhuman primates for basic research and testing.

International Action to Protect Nonhuman Primates

In view of the special role that nonhuman primates play in human health, the member states of WHO, acting collectively during the 28th and 29th World Health Assemblies, expressed their concern that primate species were being endangered by the destruction of their natural habitats, uncontrolled trade, and the lack of national and international supervision during their capture and transport. Resolutions WHA28.83 and WHA29.67 were approved requesting the director-general to: "Facilitate the exchange of both resources and technology between all countries concerned and, with the help of other interested international organizations as appropriate, to make expert advice available to countries, at their request, on the conservation, breeding, and utilization of nonhuman primates."

APPENDIX: ORGANIZATIONS AND SOURCES OF DOCUMENTS

AATA (Animal Transport Association). Administrator, Cherie Derouin, P.O. Box 797095, Dallas, TX 75379-7095, or Tim Harris, c/o Harris Associates, Ltd., Crab Hill Farm, South Nutfield, Redhill, Surrey RH1 5NR England.

Academy of Surgical Research. "A Policy Statement: The Use of Animals in Surgical Research" c/o Animal Welfare Chairman M. Michael Swindle, Medical University of South Carolina, 171 Ashley Avenue, Charleston, SC 29425.

Animal (Scientific Procedures) Act. (UK). 1986. For information: The Secretary of State, The Home Office, Cruelty to Animals Inspectorate, 50 Queen Anne's Gate, London, SW1 H9AT. Copies of the Council of Europe Convention may also be obtained. To Purchase: H.M.S.O. Publications Center, 51 Nine Elms Lane, London SW8 5DR.

APHIS (Animal and Plant Health Inspection Service). Deputy Administrator for Regulatory Enforcement and Animal Care, APHIS, USDA, Room 208, Federal Building, 6505 Belcrest Road, Hyattsville, MD 20782. Telephone: 301/436-8323.

APV (Association of Primate Veterinarians). Secretary, Joseph Bielitzki, DVM, Yerkes Primate Research Center, Emory University, Atlanta, GA 30322. Telephone: 404/727-3394.

ASLAP (American Society of Laboratory Animal Practitioners). Secretary-Treasurer, Bradford S. Goodwin, Jr., University of Texas Medical School, 6431 Fannin St., Room 1132, Houston, TX 77030. Telephone: 713/792-5127.

ASP (American Society of Primatologists). President Richard Rawlins, Ph.D., Department of Obstetrics and Gynecology, Rush Presbyterian-St. Lukes Medical Center, Chicago, IL 60612. Telephone: 312/942-2152.

AVMA (American Veterinary Medical Association). 930 N. Meacham Road, Schaumburg, IL 60196. Telephone: 1-800/248-2862.

AWIC (Animal Welfare Information Center). National Agriculture Library, attn: AWIC, 10301 Baltimore Blvd., Room 305, Beltsville, MD 20705-2351. Telephone: 301/344-3212.

CCAC (Canadian Council on Animal Care). "Guide to the Care and Use of Experimental Animals." Canadian Council on Animal Care, 1000-151 Slater Street, Ottawa, Ontario, Canada K1P 5H3. Telephone: 613/238-4031.

CDC (Centers for Disease Control). "Morbidity and Mortality Weekly Report." Available by subscription from the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402. Telephone: 202/783-3238.

CIOMS (Council for International Organizations of Medical Sciences). Address orders for "International Guiding Principles for Biomedical Research Involving Animals," 1985, to: the World Health Organization, Distribution and Sales, 1211 Geneva 27, Switzerland (ISBN-92-9036-019-4). Publications may also be obtained directly from the World Health Organization (see WHO).

CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora). Federal Wildlife Permit Office, U.S. Fish and Wildlife Service, Washington, D.C. 20240. Telephone: 703/235-1937. For CITES permits in Canada contact the Canadian Wildlife Service, Ottawa, Ontario K1A OH3. For information about other permits call (613) 957-5657.

COE (Council of Europe). U.S. sales agent for COE publications: Manhattan Publishing Company, 1 Croton Point Avenue, P.O. Box 650, Croton, NY 10520.

FWS (Fish and Wildlife Service). (See CITES).

IATA (International Air Transport Association). "Live Animal Regulations," 17 ed. July 1, 1990. From North, Central, and South America, Asia, Australia, and the Pacific: Publications Assistant, IATA, 2000 Peel Street, Montreal, Quebec, Canada H3A R4. From Europe, Africa, and the Middle East: Publications Assistant, IATA, Route de l'Aeroport 33, P.O. Box 672, CH-1215 Geneva 15 Airport, Switzerland.

ILAR (Institute of Laboratory Animal Resources). National Research Council, 2101 Constitution Avenue, N.W. Washington, D.C. 20418. Telephone: 202/334-2590.

IPS (International Primatological Society). W. Richard Dukelow, Ph.D., President, Endocrine Research Center, Michigan State University, East Lansing, MI 48824. Telephone: 517/355-7475.

NABR/FBR (National Association for Biomedical Research/Foundation for Biomedical Research). 818 Connecticut Avenue, N.W. Washington, D.C. 20006. Telephone: 202/457-0654.

NAL (National Agriculture Library). For information contact: NAL, 10301 Baltimore Blvd., Beltsville, MD 20705-2351. Tel: 301/344-4479.

NIH (National Institutes of Health). NIH Office of Animal Care and Use. Building 12A, Room 4007, NIH, Bethesda, MD 20892. Telephone: 301/496-5424.

NLM (National Library of Medicine). For information on specific bibliographies contact: National Library of Medicine, Reference Section, 8600 Rockville Pike, Bethesda, MD 20894. Copies of specific Current Bibliographies in Medicine reports ($3.00) should be ordered from the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402. Telephone: 202/496-6095.

PAHO (Pan American Health Organization). Regional Office of the World Health Organization for the Americas. Coordinator, Veterinary Public Health, 525 23rd St. NW, Washington, D.C. 20037. Telephone: 202/861-3190.

SCAW (Scientists Center for Animal Welfare). 4805 St. Elmo Avenue, Bethesda, MD. Telephone: 301/654-6390.

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CHAPTER 3

Taxonomy

Robert A. Whitney

I. INTRODUCTION

The most concise definition of the order Primates in its present evolutionary state is probably that of St. George Mivart (1873): "The group may be thus defined: Unguiculate claviculate placental mammals, with orbits encircled by bone; three kinds of teeth, at least at one time of life; brain always with a posterior lobe and calcarine fissure; the innermost digits of at least one pair of extremities opposable; hallux with a flat nail or none; a well-developed caecum; penis pendulous, testes scrotal; always two pectoral mammae."

Primate taxonomy slowly changes as data accumulate and consensus grows. Recent changes in primate taxonomy are illustrated by the case of the tarsier, a nocturnal insect eater of southeast Asia formerly classified as a monotypic family (Tarsiidae) in the primate suborder Prosimii and now classified as a separate, single-family suborder, the Tarsioidea (Napier and Napier, 1985). Similarly, the tree shrews, squirrel-like mammals from southeast Asia, were classified for some time as the prosimian family Tupaiidae, but are now classified as a separate order, Scandentia, not as primates (Napier and Napier, 1985). Although such fundamental changes are not common, primatologists inevitably differ on the number of primate genera and species that should be recognized. This chapter follows the comprehensive classification found in "The Natural History of the Primates" by Napier and Napier (1985), with a few exceptions as noted in the text. Napier and Napier largely follow Corbet and Hill's "A World List of Mammalian Species" (1980). However, they approvingly quote Elwyn Simons's dictum "there is no such thing as a correct classification." In addition to the 1985 book by Napier and Napier, their earlier "A Handbook of Living Primates" (1967), still available in many libraries, remains valuable, although it reflects former classification systems.

In this chapter, reference to a species as endangered refers to inclusion in Appendix I to the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)

NONHUMAN PRIMATES IN BIOMEDICAL RESEARCH: BIOLOGY AND MANAGEMENT

as of February 20, 1990 (U.S. Fish and Wildlife Service, 1990a) or inclusion as endangered in the list of species determined by the U.S. Fish and Wildlife Service to be endangered or threatened as of April 15, 1990 (U.S. Fish and Wildlife Service, 1990b). All primate species are on the CITES Appendix II, "Threatened," unless named on Appendix I ("Endangered"). Endangered species are seldom used in biomedical or behavioral research. For information on regulations, see Chapter 2.

Before discussing the higher order classifications of primates, a few words are in order about the tree shrews (order Scandentia), formerly classified as the prosimian family Tupaiidae. These squirrel-like creatures of southeast Asia were formerly viewed as the most primitive of primates. They are primarily insectivorous. One species, the nocturnal, feather-tailed Ptilocercus (*Ptilocercus lowii*) is classed in a separate subfamily Ptilocercinae. The 17 or more other species constitute the subfamily Tupaiinae, of which the species most used in biomedical research is the common tree shrew, *Tupaia glis*. Its adult weight is about 170 g. Generally less arboreal than their name would suggest, most tree shrews obtain food by rooting through leaf litter on the forest floor. These animals reproduce rapidly and often. However, like most prosimians, they have been reported as difficult to breed successfully in captivity (Bearder and Pitts, 1987). Hand rearing has been reported as very successful.

II. HIGHER ORDER CLASSIFICATION

Order Primates
Suborder Prosimii: Prosimians
Suborder Tarsioidea: Tarsiers
Suborder Anthropoidea: Anthropoids

The two main suborders of the order Primates are the Prosimii ("early" or "pre-" monkeys) and the Anthropoidea ("human-like"). As mentioned, tarsiers are now classed as a separate suborder, Tarsioidea, because they share several anatomical characters with each of the two other suborders and it is unclear to which suborder they are more closely related.

Napier and Napier (1985) summarize the distinguishing features of prosimians and anthropoids as follows, with some exceptions noted:

> In general, prosimians are distinguished by having rather long noses terminating in a naked moist snout or rhinarium. The muzzle and brows are provided with prominent whiskers. Their eyes face slightly sideways and are adapted for nocturnal vision. Their ears are large and mobile. They have a toilet claw (used for self-grooming) on the second digit of the foot, but flat nails everywhere else. . . . They have 36 teeth with a toothcomb formed by the lower incisors and canines, and a sublingual structure for cleaning it. The mandible is in two parts, joined in the midline by cartilage. They have specialized scent glands, particularly in the genital region. These characters fit prosimians for a nocturnal existence in which the senses of smell and hearing play a more important role than that of sight.

Exceptions noted concerning the prosimian families are that the Indriidae (Indriids) have only 30 teeth; the two larger indriids, *Indri* and *Propithecus*, are diurnal; and the Lemuridae contain several diurnal genera among the larger forms, *Lemur*, *Varecia*, and *Hapalemur*. Prosimians are not the only primates with specialized scent glands; however, such glands are prominent in the prosimian lemurs (Napier and Napier, 1967). Unlike other prosimians, the lemuriform family Daubentoniiae (aye-aye) has claws on all digits except the big toe.

> Anthropoids are characterized by having short faces, dry noses, and lacking prominent whiskers. Their ears are small and virtually immobile. The eyes face forward and are adapted for diurnal vision in colour. Flat nails are found on all digits. . . . There is no toothcomb or sublingua, and the number of teeth varies from 36 in some of the platyrrhines to 32 in the catarrhines. The mandible is formed of a single bone. Specialized scent glands are found mainly in the platyrrhines. . . . Taken together, anthropoid characters suggest adaptations to a diurnal way of life in which vision plays a more important role than smell and hearing.

Exceptions to the general anthropoid characteristics are that the marmosets (Callitrichidae) have sharp, curved claws except for a flat nail on the big toe and that the owl monkey (*Aotus trivirgatus*) is nocturnal.

The features of the "in-between" suborder Tarsioidea (tarsiers) that resemble prosimian characteristics include nocturnality, large eyes, large mobile ears, and toilet claws (two on each foot instead of only one as in the prosimians). Like anthropoids, tarsiers lack the naked rhinarium and dental comb, and have a dry furry nose and upright lower incisors. Unique among primates, tarsiers have 34 teeth and the bones of the lower leg are fused, as in a rabbit.

The prosimian and anthropoid suborders each have two infraorders: the Prosimii are divided into the Lemuriformes (lemurs, indriids, and aye-aye) and Lorisiformes (lorises and galagos) and the Anthropoidea are divided into the Platyrrhini (New World monkeys) and the Catarrhini (Old World monkeys, apes, and humans). The name Platyrrhini is derived from the Greek "broad nosed" whereas Catarrhini is derived from "hook nosed."

III. THE PROSIMIANS: SUBORDER PROSIMII

A. Lemuriforms: Lemurs, Indriids, and Aye-aye

The Lemuriformes (Malagasy lemurs) are restricted to Madagascar and the Comoro Islands. All species are classified as endangered. This infraorder is divided into two superfamilies: Lemuroidea (lemurs and indriids) and Daubentonioidea (the aye-aye). Within the superfamily Lemuroidea, the family Lemuridae (with 36 teeth) contains the lemurs and the family Indriidae (with 30 teeth) contains the indriids (three genera). The family Lemuridae, finally, has two subfamilies: Lemurinae (true lemurs) and Cheirogaleinae (dwarf lemurs).

The smallest of the dwarf lemurs (and among the smallest primates) are the lesser mouse lemurs (*Microcebus*) having a weight of 60–85 g. The largest of the true lemurs is the ruffed lemur (*Varecia variegata,* sometimes called *Lemur variegata*) with a weight of 3–4 kg.

B. Lorisiforms: Lorises and Galagos (Bushbabies)

The Lorisiformes, from Asia and Africa, have only one superfamily, the Lorisoidea, and one family, the Lorisidae. However, the genera are divided between two very distinct subfamilies: the slow-moving Lorisinae (slender loris, slow lorises, potto, and angwantibo) and the quick-moving Galaginae (galagos or bushbabies). Of the Lorisinae, the slender loris (*Loris tardigradus*) and slow lorises (*Nycticebus* spp.) are Asian whereas the potto (*Perodicticus potto*) and angwantibo (*Arctocebus* sp.) are African. All the galaginae (four genera comprising six species) are African.

The lorisinae range in size from the tiny (150 g) angwantibo of West Africa to the 1600-g potto from West and Central Africa. They have not been used in any numbers in biomedical research.

The galaginae, found across central Africa, are viewed by Napier and Napier (1985) as consisting of four genera: *Galago,* bushbabies or typical galagos; *Otolemur,* the greater bushbaby or thick-tailed bushbaby; *Euoticus,* needle-nailed galagos; and *Galagoides,* Demidoff's galago or dwarf galago. However, there is much disagreement among taxonomists about classification of the galaginae (Nash *et al.,* 1989). The galaginae range in size from the Demidoff's galago (*Galagoides demidoff,* also called *G. demidovii*) to the 1200- to 1500-g thick-tailed bushbaby (*Otolemur crassicaudatus*) of southcentral Africa. The female galago has an imperforate vagina except during the breeding and birth season. Napier and Napier view the greater bushbabies as a genus with one species, *Otolemur crassicaudatus.* Others distinguish two species, *O. crassicaudatus* (large-eared) and *O. garnettii* (small-eared) (Nash *et al.,* 1989). The greater bushbaby has been used in biomedical research, as has the typical bushbaby (*G. senegalensis*), also called lesser bushbaby. It has been reported that the most robust and self-sustaining captive colonies among prosimians have been established for a few species of bushbabies (Bearder and Pitts, 1987).

IV. TARSIERS: SUBORDER TARSIOIDEA, FAMILY TARSIIDAE

Tarsiers (*Tarsius* spp.) of the Philippines and southeast Asia are rat-sized nocturnal animals that are highly specialized. These animals have occasionally been used in biomedical research. Adult weight is about 120 g; they are about the same size as Demidoff's galago, but heavier.

Their eyes are very large; the tail is much longer than the body; and the tibia and fibula are fused in the lower third of their length. The three species are the Philippines tarsier (*T. syrichta*); Horsfield's tarsier (*T. bancanus*), found in Borneo and Sumatra; and the spectral tarsier (*T. spectrum*), found in Sulawesi. Musser and Dagosto (1987) argue that a small, rare Sulawesian tarsier is a separate species (*T. pumilus*) instead of a subspecies of *T. spectrum.*

V. TRUE PRIMATES: SUBORDER ANTHROPOIDEA

A. New World Monkeys: Superfamily Ceboidea

A simple list of contrasts shown in Table I highlights the most marked differences between the Platyrrhines (New World monkeys) and the Catarrhine monkeys. New World monkeys require dietary vitamin D_3 (animal source) in the absence of ultraviolet light (see Chapter 11 nutrition and feeding). Insects are an important part of the natural diet of most species. There are two families of New World monkeys: the Callitrichidae (marmosets, pygmy marmoset, and tamarins) have 32 teeth and sharp, curved claws on hands and feet except for a flat nail on the big toe whereas the much more varied Cebidae (cebid monkeys) have 36 teeth and nails on hands and feet. One rare and endangered callitrichid species, Goeldi's marmoset (*Callimico goeldii*) of the Amazon headwaters from Colombia to Bolivia, has 36 teeth like the Cebidae and is now classified as the sole member of the subfamily Callimiconinae, leaving the other Callitrichidae in the subfamily Callitrichinae.

Within the Callitrichinae, the mandible is a marked distinguishing factor between marmosets and tamarins. Marmosets and pygmy marmosets have a V-shaped mandible with long incisors and short canines whereas tamarins and golden-lion tamarins (and Goeldi's marmoset as well) have a U-shaped mandible with short incisors and long canines (Napier and Napier, 1985).

A fundamental point about New World monkeys is that some species, especially squirrel and spider monkeys, possess a

TABLE I

DIFFERENCES BETWEEN PLATYRRHINE AND CATARRHINE MONKEYS

Platyrrhines	Catarrhines
Broad-nosed	Narrow-nosed
Require vitamin D_3 in the diet	Do not require vitamin D_3 in the diet
Some have prehensile tails	No prehensile tails
No cornified pads over ischial tuberosities	Some have callus over ischial tuberosities
Do not have opposable thumbs	All have opposable thumbs
Have three premolar teeth on each side	Have two premolar teeth on each side

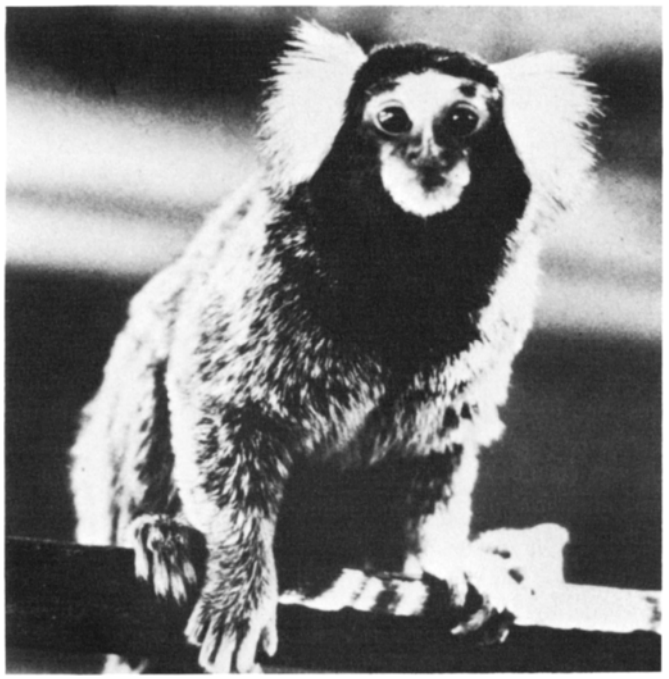

Fig. 1. *Callithrix jacchus*, common marmoset (photo from L. Davis). Courtesy of the Wisconsin Primate Research Center Audiovisual Services.

number of different strains of herpes virus that are harmless to the natural host but are lethal to other New World species. Great care must be taken to separate species in accord with the colony management principles discussed elsewhere in this volume.

1. Family Callitrichidae: Subfamily Callitrichinae

These are the most primitive New World primates. These primates range in size from the 5-inch pygmy marmosets, adult weight 150 g, to the larger tamarins weighing as much as 600 g. There are four genera. Marmosets (*Callithrix* spp.) and tamarins (*Saguinus* spp.) are often referred to globally as "marmosets," but they are quite distinct. Both genera are used in research. These animals are susceptible to measles, mumps, and other human diseases, which are often fatal to them. The *Herpes saimiri* virus may also be fatal to them, so marmosets and tamarins should never be held in the same rooms as squirrel monkeys.

The other two genera have only one species. The pygmy marmoset (*Cebuella pygmaea*) of the Upper Amazon, the smallest New World monkey, is seldom used in research. The golden-lion tamarin (*Leontopithecus rosalia*) of southeastern Brazil is one of the rarest and most gravely endangered primate species.

a. *CALLITHRIX* SPP.: MARMOSETS Classification of the *Callithrix* taxa is controverted. Hershkovitz (1977) divided 10 taxa among three species: *C. jacchus* (common marmoset) (Fig. 1), *C. argentata* (silvery marmoset), and *C. humeralifer* (black-tailed or bare-eared marmoset). Coimbra-Filho and Mittermeier (1981) divided the taxa among seven species. This discussion uses the Hershkovitz classification, followed by Napier and Napier (1985).

C. jacchus jacchus (often referred to simply as *C. jacchus*), from the Amazon Basin in eastern Brazil, is the most widely used of the Callitrichidae in biomedical research and has the greatest potential as a general laboratory primate (Hearn, 1987). Marmosets are very fecund; females bear their young twice a year and usually have twins. This primate breeds well and is adaptable in captivity. Conservation status in the wild is good, and many captive colonies are thriving, but habitat destruction is continuing. *C. jacchus jacchus* is the only *Callithrix* species available in any numbers to laboratories outside Brazil. Two other Hershkovitz *C. jacchus* subspecies are endangered: the buffy-headed marmoset (*C. jacchus flaviceps*) and the buffy tuft-eared marmoset (*C. jacchus aurita*). The Brazilian government prohibits export of all monkeys, including marmosets.

Marmosets are small monkeys. Adult body weight reports vary widely. Hearn (1987) cites a range of 350–400 g from one source and a range of 386–493 g for males and 382–600 g for

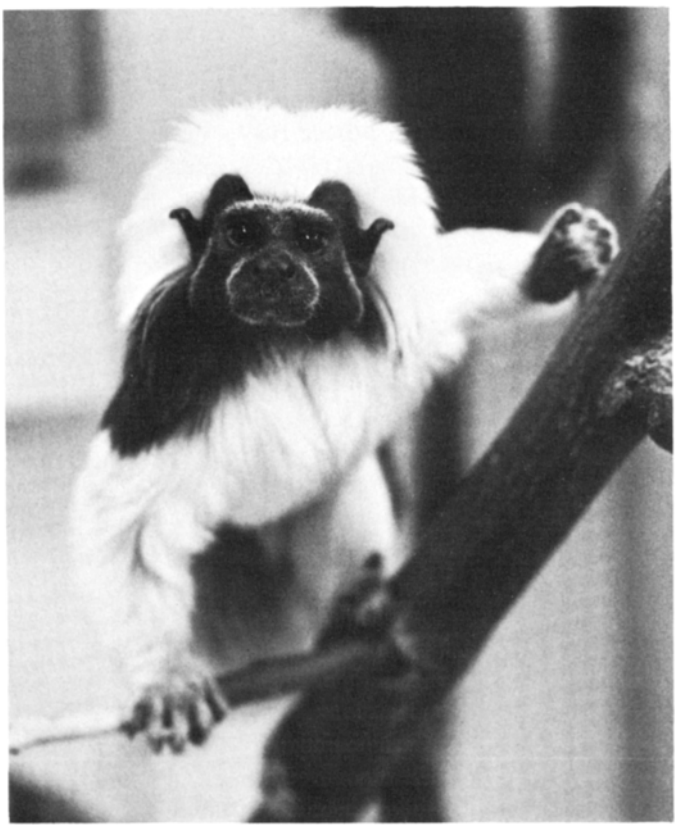

Fig. 2. *Saguinus oedipus*, cotton-top tamarin (photo from Robert Dodsworth). Courtesy of the Wisconsin Regional Primate Research Center Audiovisual Services.

Fig. 3. *Saguinus mystax*, moustached tamarin (photo courtesy of NIH).

nonpregnant females from another. *C. jacchus jacchus* has marbled black and gray body fur; its tail has black and gray rings. Bushy white tufts cover the ears.

b. *SAGUINUS* SPP.: TAMARINS. Tamarins range widely through South American tropical forest areas, reaching Panama and Costa Rica. Tamarins and marmosets overlap geographically very little. Tamarins are slightly larger than marmosets: adult weight ranges from 200 g to more than 500 g. The names of the 11 species often reflect their striking colors and markings. The cotton-top tamarin, *S. oedipus* (Fig. 2), used in colon cancer research is now endangered. Other endangered species are *S. bicolor* (pied or bare-faced tamarin) and *S. leucopus* (white-footed tamarin). The most widely used species today are probably the mustached tamarin (*S. mystax*) (Fig. 3), in hepatitis A research. The white-lipped (red-bellied) tamarin (*S. labiatus*), and the saddleback tamarin (*S. fuscicollis*). *S. labiatus* and *S. fuscicollis* appear to breed year round in captivity (Hearn, 1987).

2. Family Cebidae

This family contains all other New World monkeys. It is divided into seven subfamilies shown in Table II. The first four families (owl monkeys, titis, sakis and uakaris, and squirrel monkeys) do not have prehensile tails, unlike the "hand-tailed" New World monkeys of the other three families (capuchin, howler, spider, and woolly monkeys) (Sanderson, 1957).

TABLE II

SUBFAMILIES OF CEBIDAE MONKEYS

Subfamily	Genus and species	Common name	Number of species
Aotinae	*Aotus trivirgatus*	Owl monkeys	1
	Aotus spp.	—	9[a]
Callicebinae	*Callicebus* spp.	Titis	3
Pitheciinae	*Pithecia* spp.	Sakis	2
	Cacajao spp.	Uakaris	3
	Chiropotes spp.	Bearded sakis	2
Saimirinae	*Saimiri* spp.	Squirrel monkeys	2
Cebinae	*Cebus* spp.	Capuchin monkeys	4
Alouattinae	*Alouatta* spp.	Howler monkeys	6
Atelinae	*Ateles* spp.	Spider monkeys	4
	Brachyteles arachnoides	Woolly spider monkey	1
	Lagothrix spp.	Woolly monkeys	2

[a]See Hershkovitz (1983).

Endangered species are the *Cebus capucinus* (white-throated capuchin), *Saimiri oerstedii* (red-backed squirrel monkey), *Cacajao* spp. (uakaris), *Chiropotes albinasus* (white-nosed bearded saki), *Alouatta palliata* (mantled howler monkey), *Ateles geoffroyi frontatus* and *Ateles geoffroyi panamensis* (black-handed spider monkey), *Brachyteles arachnoides* (woolly spider monkey), and *Lagothrix flavicauda* (yellow-tailed woolly monkey or Hendee's woolly monkey).

a. AOTINAE: OWL MONKEY. The only nocturnal anthropoid primate, the owl monkey (also called night monkey and dourocouli), is widely distributed throughout the rain forest areas of South America. Owl monkeys have long been classified as a single phenotypic species, *Aotus trivirgatus* (Fig. 4), but there is considerable variation in their genotypes. Hershkovitz (1983) has suggested there may be as many as nine species. The abstract of his article is:

> The nine allopatric species of *Aotus* recognized represent two natural groups distinguished by karyotype, color, and pelage patterns. Correlated with these group characters are reported differences in serum proteins and degrees of susceptibility or immunity to experimental infection with malarial parasites. The primitive gray-neck species group of *Aotus* contains *A. nancymai* (new species), *A. lemurinus* (with subspecies *lemurinus* and *griseimembra*), *A. trivirgatus*, and *A. vociferans*. The derived red-neck group contains *A. nancymai* (new species), *A. miconax*, *A. infulatus*, and *A. azarae* (with subspecies *azarae* and *boliviensis*). Only the two new species are described but a key to the species and subspecies gives the diagnostic characters of each. The gray-neck group occurs almost entirely north of the Amazon, the red-neck group almost entirely south. The distributional exceptions are enclave populations resulting from river bend cutoffs. Formation of an enclave population of *A. nancymai* is discussed and available information on the biology of this species is reported.[1]

[1] From *Am. J. Primatol.* **4**, 209. Copyright © 1983. Reprinted with permission of Wiley-Liss, a division of John Wiley and Sons, Inc.

Fig. 4. *Aotus trivirgatus*, owl monkey (photo from R. Fountaine). Courtesy of the Wisconsin Primate Research Center Audiovisual Services.

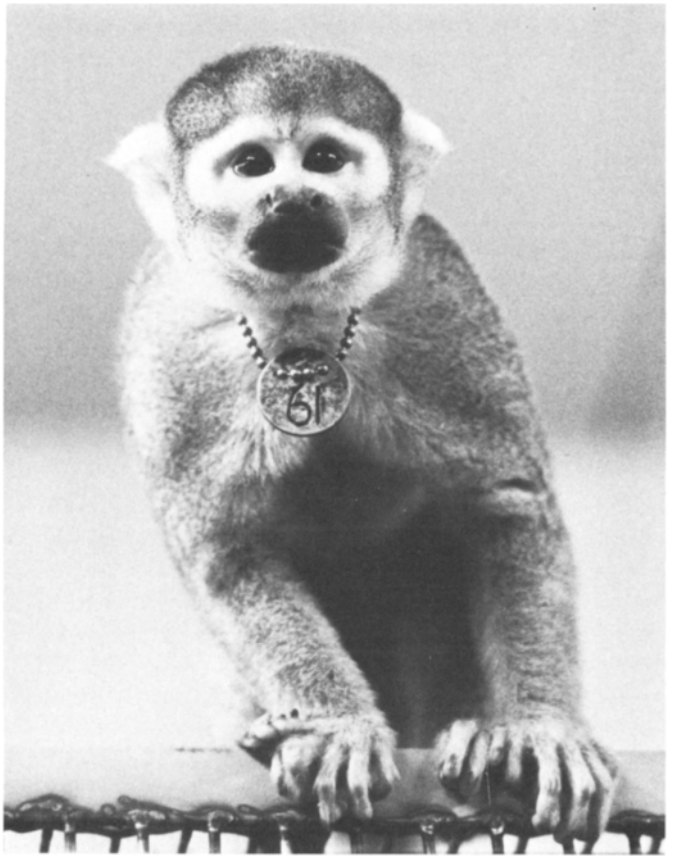

Fig. 5. *Saimiri sciureus*, squirrel monkey (photo from Robert Dodsworth). Courtesy of the Wisconsin Regional Primate Research Center Audiovisual Services.

Dixson (1987) strongly recommends determining the phenotype of captive animals and, if possible, karyotyping them before breeding.

In biomedical research, owl monkeys are best known for their crucial role in antimalarial drug development, but they are also used in immunology and vision research and other studies. They weigh from 900 to 1200 g. The name "trivirgatus" refers to three dark strips separating white crescents above the large eyes.

b. CALLICEBINAE: TITIS. Titis, *Callicebus* spp., were formerly classed in the family Aotinae, but are diurnal and do not have enlarged eyes. These monkeys are smaller than owl monkeys, weighing about 600 g. The three species are the masked titi (*C. personatus*), dusky titi (*C. moloch*), and widow monkey or white-handed titi (*C. torquatus*).

Hershkovitz (1990) has proposed a distinction of 13 *Callicebus* species in four species groups: the *modestus* group, *donacophilus* group, *moloch* group (including *C. moloch* and *C. personatus*), and *torquatus* group. The *modestus* group is described as externally similar to the *moloch* group, and the *donacophelus* group is intermediate between them but nearer the *moloch* group.

c. PITHECIINAE: SAKIS, UAKARIS, AND BEARDED SAKIS. These medium-sized monkeys are little used in research. Uakaris (*Cacajao* spp.) are endangered. All three genera comprise animals of unusual appearance. Sakis (also called sakiwinkis) have long, coarse, shaggy coats, with the neck hair growing forward from the nape. They have the platyrrhine nose at its broadest, and in the male *Pithecia pithecia* this black nose is surrounded by a white mask. The bearded sakis (*Chiropotes* spp.) have dense, woolly hair and a bouffant "top knot." Napier and Napier (1985) refer to two of the three Uakari species (*Cacajao calvus* and *C. rubicundus*) as "truly grotesque in appearance." They have bare crimson faces, bald heads, and long shaggy coats.

d. SAIMIRINAE: SQUIRREL MONKEYS. This single-genus family was formerly classed in the Cebinae with capuchin monkeys. Napier and Napier (1985) divide squirrel monkeys into two species: *Saimiri sciureus* and *S. oerstedii*. The common squirrel monkey *S. sciureus* (Fig. 5), much used in biomedical research for many years, is widely distributed through tropical rain forests of South America, except for southeastern Brazil; they are particularly prevalent throughout the Amazon Basin. The red-backed squirrel monkey *S. oerstedii,* found in Costa Rica and Panama, is an endangered species.

Squirrel monkeys have short, dense hair, varying in color, with an adult weight of 500–1000 g. Variations in shape and appearance of the area around the eyes have long been used to characterize their geographical origin. The "Roman arch" face (forehead coloration like that of crown) is characteristic of animals exported from Iquitos, Peru. The "gothic arch"

Fig. 6. Cebus apella, black-capped capuchin monkey, young adult (photo from Frans de Waal). Courtesy of the Wisconsin Regional Primate Research Center Audiovisual Services.

face (forehead with whitish arching bands above each eye) is characteristic of animals from Leticia, Colombia, or from Guyana. Hershkovitz (1984) has presented the case for a reclassification of genus *Saimiri* into two groups based on these facial characteristics and other factors. In his system, the "Roman" type is *S. boliviensis* of upper Amazonia (two subspecies) whereas the "Gothic" type contains *S. sciureus* (four subspecies), *S. oerstedi* (two subspecies), and *S. ustus*, making four species in all.

Thorington (1985) argues for recognition of only two species, *S. sciureus* and *S. madeirae*. In this classification, *S. sciureus* has four subspecies: *S. sciureus sciureus, S. sciureus cassiquiarensis, S. sciureus boliviensis*, and *S. sciureus oestedii*. *S. madeirae* is a species found along the Rio Madeira in Brazil, having a gray crown and nearly naked ears.

Squirrel monkeys used in biomedical research should be karyotyped. Those from Peru, Colombia, and Guyana have, respectively, 10, 12, and 14 acrocentric chromosomes; *S. oerstedi* from Costa Rica and Panama matched the Peruvian karyotype of 10, and the "Bolivian-type" matched the Colombian (Dukelow and Asakawa, 1987). Hershkovitz (1984) notes that "As yet, there is no reason to believe that the karyotype known from one or a few individuals of a taxon is essentially the same for all individuals throughout the geographic range of that taxon."

e. CEBINAE: CAPUCHIN MONKEYS. These are wide-ranging, medium-sized monkeys. The adults weigh 3–5 kg. Although prehensile, the tail does not have naked skin at the tip as seen in spider monkeys. The four species are the black and white *Cebus capucinus* or white-throated capuchin (an endangered species); the brown and white *C. albifrons* or white-fronted capuchin; the *C. nigrivittatus* or weeper capuchin, seldom used in research; and the uniquely tufted *C. apella* (Fig. 6), black-capped capuchin, stocky with brown sideburns (the "organ grinder's monkey" of years past).

f. ALOUATTINAE: HOWLER MONKEYS. The wide-ranging and large howlers have not done well in captivity and have seldom been used in biomedical research. The adults weigh from 6 to 8 kg. The six *Alouatta* spp. are *A. belzebul* (black and red), *A. caraya* (black), *A. fusca* (brown), *A. palliata* (mantled), *A. seniculus* (red), and *A. villosa* (Guatemalan). CITES lists "*Alouatta palliata* (=*villosa*)" as endangered (U.S. Fish and Wildlife Service, 1990a). The U.S. Fish and Wildlife Service (1990b) uses the common name "howler monkey" for a similar listing.

g. ATELINAE: SPIDER MONKEYS, WOOLLY SPIDER MONKEY, AND WOOLLY MONKEYS. The spider monkeys are the four *Ateles* spp.: *A. pansicus* (black), *A. belzebuth* (long-haired), *A. fusciceps* (brown-headed), and *A. geoffroyi* (black-handed). These monkeys lack a thumb and are pot-bellied with spidery limbs. The females have pendulous external genital labia that resembles a penis. Adults may weigh up to 7 kg. *A. geoffroyi frontatus* (Costa Rica, Nicaragua) and *A. geoffroyi panamensis* (Costa Rica, Panama) are endangered.

The woolly spider monkey (*Brachytels arachnoides*) or muraqui is a highly endangered species of the Brazilian Atlantic forest. They are large, with an adult weight of 12–15 kg. Like the spider monkeys, they lack a thumb and have long, lanky limbs; like the woolly monkey, they have short plushy fur and a rounded head.

The two woolly monkey species are *Lagothrix lagothricha* or common woolly monkey (Humboldt's woolly monkey) of the middle and upper Amazon basin and the endangered *L. flavicauda* (yellow-tailed woolly monkey) found only in the Peruvian Andes. Woolly monkeys are large and may grow to 10 kg.

B. Old World Monkeys: Superfamily Cercopithecoidea, Family Cercopithecidae

Old World monkeys all lack prehensile tails and have some degree of callus over the ischial tuberosities, an apparent

adaptation for sitting. Many have cheek pouches, and all have a narrow nasal septum. All that have thumbs show opposability.

Old World monkeys are divided into two subfamilies: the Cercopithecinae and the Colobinae. The nine genera of the Cercopithecine subfamily fall roughly into three general groups: the African long-tailed monkeys (guenons, talapoins, patas monkeys, and Allen's swamp monkey), the mangabeys, and the closely related macaques, baboons, and baboon-like monkeys. The seven genera of the Colobine subfamily are colobus monkeys, langurs, and other leaf eaters.

1. Subfamily Cercopithecinae

a. *CERCOPITHECUS* SPP.: GUENONS. Guenons are primarily arboreal, colorful primates with green, yellow, and black fur colors. Most of the 20 species and more than 60 subspecies have distinctive, brilliantly colored patterns both on the front and on the back that serve to prevent hybridization. Guenons are the most common African monkeys, found throughout sub-Saharan Africa in many environments. They are divided among eight species groups.

The "*C. aethiops* group," commonly used in research, is now viewed as a single species, *C. aethiops* (Fig. 7). Most commonly called the "African green" monkey in the biomedical research community, it is also called savanna monkey, and subspecies are sometimes referred to as grivet, vervet, and tantulus monkey.

The African greens range from Senegal to the Sudan and Ethiopia and southward. They are becoming adapted to a ground-living way of life, eating savanna grasses and seeds (Napier and Napier, 1985). There are also substantial wild populations of *C. aethiops* on several West Indian islands, including St. Kitts and Nevus; the ancestors of most of these populations were brought to the West Indies in the 17th century.

The ischial callosities of guenons are much smaller than those of macaques and baboons. There is no sexual swelling at estrus. The male scrotum is typically blue. Adult males weigh 3–6 kg and adult females weigh 2–4 kg.

Endangered *Cercopithecus* are *C. diana, C. lhoesti, C. erythrogaster,* and *C. erythrotis.*

b. *MIOPITHECUS* SPP.: TALAPOINS. *Miopithecus talapoin* (the "Southern" talapoin) and the still formally unnamed "Northern" talapoin of western central Africa are closely related to guenons. However, these yellowish gray relatives are much smaller (adult weight usually less than 1 kg), and they have a sexual swelling. Talapoins have been useful in reproductive research but are still difficult to breed in the laboratory.

c. *ERYTHROCEBUS PATAS*: PATAS MONKEYS. The patas monkey (Fig. 8) is a ground-living species inhabiting the sub-Saharan savanna and subdesert. Slender bodies with long legs, a shaggy red coat, and a prominent white moustache characterize both sexes. The male (8–13 kg) is about twice as large as the female (4–7 kg). As with the African green, the ischial callosities are small and the scrotum is blue. The large size and tractable temperament of the patas monkey enhance its value for some types of biomedical research.

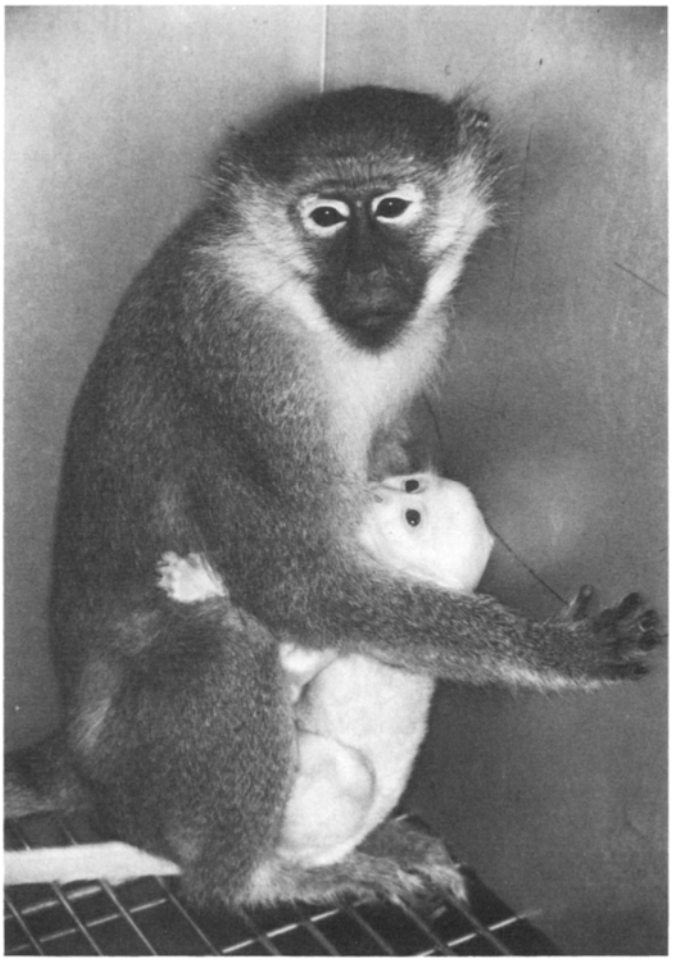

Fig. 7 *Circopithecus aethiops*, African green monkey; note albino infant (photo courtesy of David Martin, DuPont-Merck Pharmaceutical Co.).

d. *ALLENOPITHECUS NIGROVIRIDIS*: ALLEN'S SWAMP MONKEY. Allen's swamp monkey is the least known of the African long-tailed monkeys. It is sparsely distributed in the central Zaire basin. The general color is black with yellow markings. It is not used in research.

e. *CERCOCEBUS* SPP.: MANGABEYS. The mangabeys are large, slender, long-legged African monkeys. They have cheek pouches and large, flat ischial callosities, continuous in males but separate in females. Adult mangabeys and several other Old World species, including baboons and several macaque species, have a marked reddening of the skin around the genitalia and over the buttocks. In females this "sex skin" or "sexual skin" becomes highly colored and tumescent during estrus, with maximum swelling at ovulation.

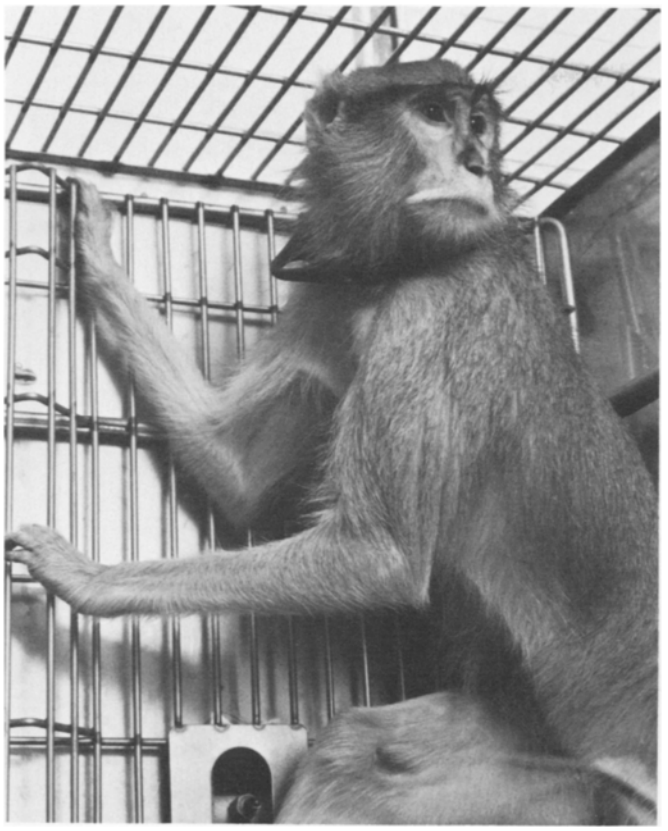

Fig. 8. *Erythroceubus patas*, Patas monkey (photo courtesy of NIH).

Fig. 9. *Cercocebus torquatus*, mangabey (photo from B. L. DePutte). Courtesy of the Wisconsin Primate Research Center Audiovisual Services.

There are two species groups, each containing two species. The *C. torquatus* group (Fig. 9), uncrested and mainly brown or black in color, ranges from west to central Africa throughout the Zaire basin; the *C. albigena* group, crested and black, has a similar distribution, reaching Uganda and Rwanda in the east.

The two species of the *C. torquatus* group are *C. torquatus* (white-collared mangabey) and *C. galeritus* (Tana River mangabey), both listed as endangered. The sooty mangabey has been viewed by some primatologists as a separate species, *C. atys*, in the *C. torquatus* group (Napier and Napier, 1967), but is now considered a subspecies: *C. torquatus atys*. These monkeys are therefore included in the listing of *C. torquatus* as endangered, although there are indications that they are relatively common in the wild. Sooty mangabeys are subject to naturally occurring leprosy (Meyers *et al.*, 1985) and may be asymptomatic carriers of the simian immunodeficiency virus (SIV) (Murphey-Corb *et al.*, 1986). They are used in leprosy vaccine development studies and AIDS research, although this is now complicated by their endangered status.

The *C. albigena* group consists of *C. albigena* (white-cheeked mangabey), with two subspecies, and *C. aterrimus* (black mangabey), also with two subspecies.

f. *MACACA* SPP.: MACAQUES. The macaques are the most widely ranging nonhuman primate genus. Napier and Napier (1985) aptly sum up their versatility: "They are found in Morocco, Algeria, Gibraltar, Afghanistan, the Indian subcontinent, China, Japan, and throughout southeast Asia, extending to the Philippines, Borneo, Sumatra, Java, and Sulawesi, and many offshore islands. They are adapted to almost any ecological niche. . . . Diet is omnivorous and extremely varied. . . . The range of macaques into northern latitudes is limited by seasonality of vegetation and short daylight periods rather than cold temperatures."

Macaques are medium to large, heavily built, and range in color through various shades of gray or brown to black. They move readily along the ground as well as in the trees and typically stuff their cheek pouches during foraging. The ischial callosities are prominent. In some species sex skin swelling during estrus is enormous. Tail length varies greatly among species, from the tailless *M. sylvanus* and *M. nigra* to the *M. fascicularis, M. radiata,* and *M. sinica,* with tails longer than head and body length (Napier and Napier, 1985). There is considerable sexual dimorphism in body size, weight, and canine size.

TABLE III

TAXONOMY OF MACAQUES

Genus and species	Common name	Locale
M. sylvanus group		
M. sylvanus	Barbary macaque or "ape"	North Africa
M. silenus	Lion-tailed macaque	Southern India
M. maurus	Moor macaque	Sulawesi
M. nemestrina	Pig-tailed macaque	Southeast Asia
M. ochreata	Sulawesi-booted macaque	Sulawesi
M. nigra	Sulawesi-crested macaque or Sulawesi black "ape"	Sulawesi
M. tonkeana	Tonkean macaque	Southeast Asia
M. fascicularis group		
M. fascicularis	Crab-eating macaque or cynomolgus monkey	Southeast Asia
M. fuscata	Japanese macaque	Japan
M. mulatta	Rhesus macaque	Central Asia
M. cyclopis	Taiwan macaque or Formosan rock macaque	Taiwan
M. sinica group		
M. sinica	Toque macaque	Sri Lanka
M. assamensis	Assamese macaque	Northeast India, Afghanistan
M. radiata	Bonnet macaque	India
M. thibetana	Tibetan stump-tailed macaque	China
M. arctoides group		
M. arctoides	Red-faced or stump-tailed macaque, bear monkey	China, Southeast Asia

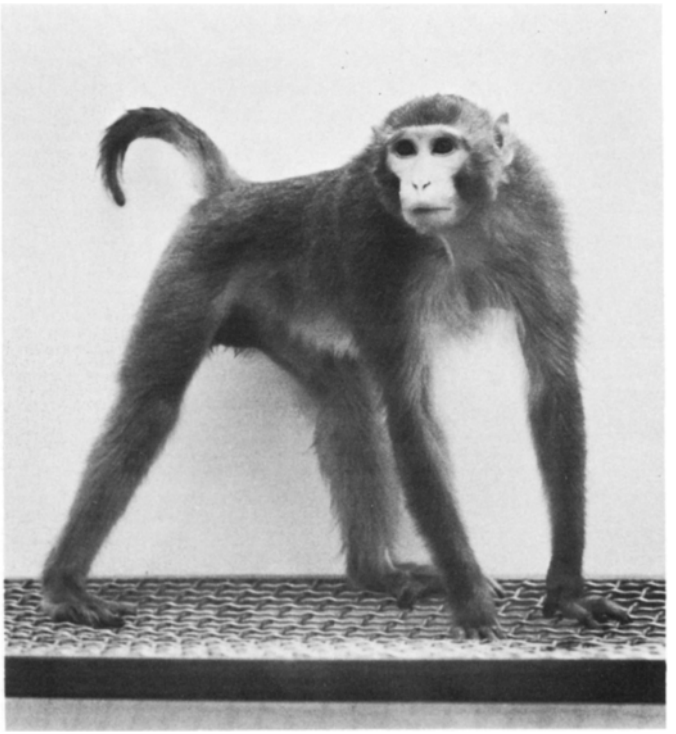

Fig. 10. Macaca mulatta, Rhesus macaque (photo from Robert Dodsworth). Courtesy of the Wisconsin Regional Primate Research Center Audiovisual Services.

Taxonomists differ on the number of species. Napier and Napier (1985) follow Fooden (1976) in dividing the genus into four species groups based on the anatomy of the male and female genitalia. This system accepts 16 species as shown in Table III.

M. silenus, the black lion-tailed macaque or wonderoo of southern India, is endangered. *M. sylvanus*, the sole African macaque, is native to North Africa and was introduced into southern Spain. *M. nigra*, the Sulawesi-crested macaque, was formerly called the Celebese black ape and was classified as *Cynopithecus niger*.

The species most commonly used in research are the rhesus, *Macaca mulatta* (Fig. 10), and the crab-eating macaque, *M. fascicularis* (Fig. 11), often called the cynomolgus monkey. Other macaques used include *M. arctoides* (Fig. 12), the stump-tailed macaque, one of the least aggressive macaques, and *M. nemestrina* (Fig. 13), the pig-tailed macaque. The rhesus is distributed across most of central Asia from Afghanistan to China. Adult male weight is 6–11 kg and female weight is 4–9 kg. The range of the cynomolgus is confined to southeast Asia, including the Philippines.

g. *PAPIO* SPP.: BABOONS. Although a distinction is frequently made between the hamadryas or sacred baboon and the four "savanna" species or "*P. cynocephalus* group," Napier and Napier (1985) simply list baboons as five species: *Papio papio* (Guinea baboon), *P. anubis* (olive baboon), *P. cynocephalus* (yellow baboon), *P. ursinus* (chacma baboon), and *P. hamadryas* (hamadryas or sacred baboon).

Baboons are widely distributed throughout sub-Saharan Africa, replacing each other geographically from Senegal and Sudan to the Cape of Good Hope except for the main forested areas. In many areas they are regarded as a pest because of damage to crops. The face is long, with prominent muzzle and jaws. *P. papio*, the Guinea baboon, is found only in extreme west Africa. The easternmost species, hamadryas, extends from Ethiopia into the extreme southwestern tip of the Arabian peninsula. The male hamadryas has a distinctive flesh pink face, but the five species all differ in coat color. *P. papio* is reddish; *P. anubis* (Fig. 14) is dark olive-gray; *P. ursinus* is dark brown, almost black; *P. cynocephalus* is yellowish; and *P. hamadryas* (Fig. 15) is silver gray with a long-haired shoulder cape (Napier and Napier, 1985). In all species there is marked sexual dimor-

Fig. 11. Macaca fascicularis, cynomolgus (crab-eating) macaque (photo from Robert Dodsworth). Courtesy of the Wisconsin Regional Primate Research Center Audiovisual Services.

phism; the male is approximately twice the size of the female. Adult males weigh 22–30 kg and females weigh 11–15 kg.

The four savanna species are promiscuous and have matrilineal troops of both sexes, usually 40 to 80 animals but occasionally up to 200. However, the hamadryas baboon forms troops consisting of one or two males with several females and their offspring. As with macaques, baboons have strict social hierarchies with continual jockeying for position.

h. *MANDRILLUS* SPP.: MANDRILL AND DRILL. Both of these large, baboon-like species are endangered. Napier and Napier (1985) refer to the mandrill as *Mandrillus sphinx* and the drill as *M. leucophaeus*, whereas CITES lists them as *Papio sphinx* and *Papio leucophaeus*, noting the name *Mandrillus* parenthetically (U.S. Fish and Wildlife Service, 1990a). These animals are forest dwellers of western central Africa. The face of the male mandrill is brilliantly colored: red nose, blue nasal swellings, and yellow to orange cheek whiskers. Drills have black faces. Males of both species have brightly colored genitalia.

i. *THEROPITHECUS GELADA*: GELADA. Found only in mountain grasslands in Ethiopia, geladas are the most terrestrial nonhuman primates. They are large, with marked sexual dimorphism. The coat is dark brown to black. The face is rounder than that of other baboon group species. Adult males have a mane and canine teeth larger than those of the great cats. Both sexes have an area of pink skin on the chest; in females this is surrounded by raised vesicles that resemble a necklace.

2. Subfamily Colobinae

These leaf eaters of Africa and southeast Asia are usually large monkeys (many adult males weigh more than 20 kg) with long tails and often with unusual noses. Most members of this subfamily are rather rare, and a number of species are endangered. There has been little use in biomedical research. Colobines have a unique stomach, consisting of a succession of lobes or pouches. They do not have cheek pouches. Tall, pointed cusps on their molar teeth are well adapted for slicing up mature leaves. In the *Colobus* spp. and *Procolobus* sp., the thumb is merely a tubercle.

Napier and Napier (1985) divide the colobines into seven genera: *Colobus* spp., colobus monkeys of Africa; *Procolobus*

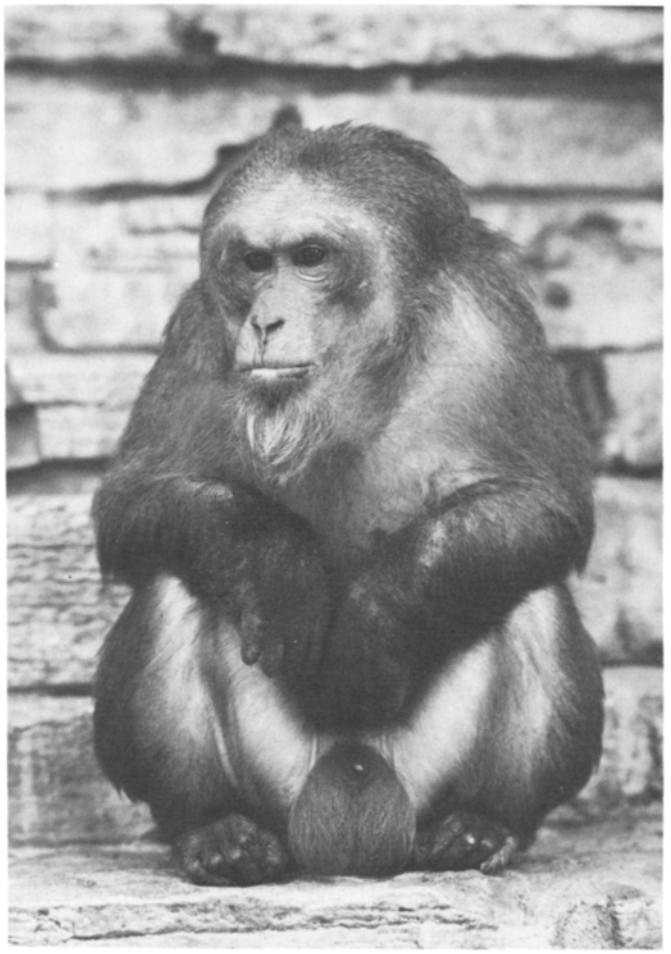

Fig. 12. Macaca arctoides, stump-tailed macaque (photo from Robert Dodsworth). Courtesy of the Wisconsin Regional Primate Research Center Audiovisual Services.

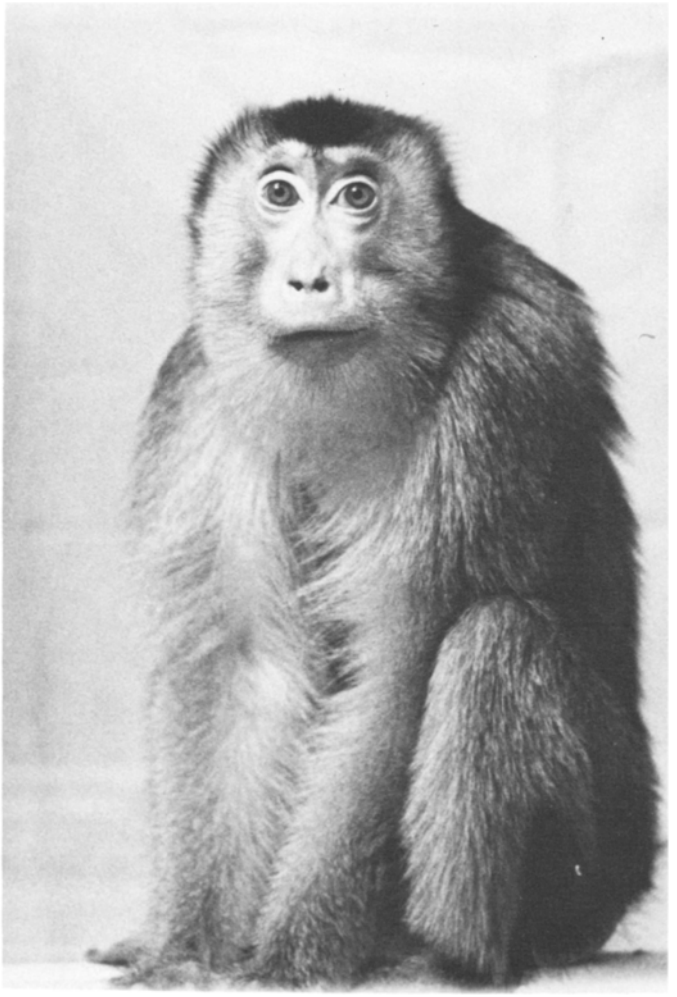

Fig. 13. Macaca nemestrina, pig-tailed macaque, female (photo from Martin Maurer). Courtesy of the University of Washington Regional Primate Center.

sp., the olive colobus of Africa (*P. verus*); *Presbytis* spp., langurs or leaf monkeys of Asia; *Pygathrix* sp., the douc langur or douc of Laos and Vietnam (*P. namaeus*); *Rhinopithecus* spp., snub-nosed langurs of China and North Vietnam; *Simias* sp., the Mentawai Islands snub-nosed monkey or pig-tailed leaf monkey (*S. concolor*) of Sumatra; and *Nasalis* sp., the proboscis monkey (*N. larvatus*) of Borneo.

There are two groups of colobus, the black and white colobus group of four species and the red colobus group of two species. Of the black and white group, *C. satanas,* the black colobus, is endangered; of the two species in the red colobus group, *C. kirkii* is endangered, as are two *C. badius* subspecies.

Procolobus verus, the olive colobus sometimes called *Colobus verus,* is also endangered.

Presbytis spp., the langurs, number 16 species, of which four are endangered: *P. entellus,* the entellus or Hanuman langur; *P. geei,* the golden langur; *P. pileata,* the capped langur; and *P. potenziana,* the Mentawai Island leaf monkey.

The four remaining colobine genera are all endangered.

C. Apes and Humans: Superfamily Hominoidea

Napier and Napier (1985) briefly distinguish the Hominoidea families as:

> The Hominoidea (gibbons, great apes, and man) are characterized by a broad chest and the absence of a tail. Within the superfamily, the gibbons (family: Hylobatidae) possess ischial callosities, a character which they share with the Old World monkeys. On the other hand, they share with the great apes (family: Pongidae) the long arms and short legs that reflect their suspensory activities in the trees. The great apes are distinguished from the gibbons by their much greater bulk. They resemble man in lacking ischial callosities and in the cusp pattern of the lower molar teeth—the Y-5 pattern.

Lacking ischial callosities, the great apes build nests for sleeping.

1. Family Hylobatidae: *Hylobates* spp. (Gibbons and Siamangs)

Primate taxonomists disagree on classifying the "lesser apes" as one genus, *Hylobates,* including both gibbons and

Fig. 14. *Papio anubis*, olive baboon (photo from Maria Lang). Courtesy of the Biologic Resources Laboratory, University of Illinois at Chicago.

siamangs, or as two genera: *Hylobates* (gibbons) and *Symphalangus* (siamangs). Napier and Napier (1985) accept a single genus with six species, in which the siamang is *Hylobates syndactylus* instead of *Symphalangus syndactylus*. Irrespective of this issue, all species of gibbons and siamangs are endangered.

Gibbons are small, tailless apes of southeast Asia with dense, shaggy hair and brachiate locomotion. They are almost wholly arboreal. Adult male weight seldom exceeds 7 kg. The best known species, *H. lars*, the white-handed gibbon, is either black or buff irrespective of sex, whereas three other species are sexually dichromatic, with the males being black and the females buff, golden, brown, or gray. Small numbers of gibbons have been used in biomedical research.

The siamang is jet black and larger than gibbons, weighing more than 10 kg. Its voice has both a high-pitched bark and a low booming sound produced when an air sac in the throat is inflated.

2. Family Pongidae: Great Apes (Orangutan, Chimpanzee, and Gorilla)

a. *PONGO PYGMAEUS*: ORANGUTAN. A single-species genus, the orangutans of Borneo and Sumatra are gravely endan-

Fig. 15. *Papio hamadryas*, Hamadryas baboon (photo courtesy of David Martin, DuPont-Merck Pharmaceutical Co.).

Fig. 16. *Pan troglodytes*, chimpanzee (photo courtesy of Yerkes Regional Primate Center, Emory University).

gered. These animals are large; adult male weight is about 77 kg and female weight is about 37 kg. The coarse hair is reddish brown, darker in the Borneo subspecies and lighter and longer in the Sumatran. They are almost wholly arboreal.

b. *PAN* SPP.: CHIMPANZEES AND PYGMY CHIMPANZEES. *Pan troglodytes* (Fig. 16), the common chimpanzee, inhabits a wide geographical zone across equatorial Africa, but their populations in many countries in that area have been devastated by hunting and the encroachment of agriculture. *Pan troglodytes* have been classified by the U.S. Fish and Wildlife Service (1990b) as endangered in the wild and threatened in captivity.

Adult chimpanzees are large (male average weight is 50 kg; female weight is 40 kg) powerful animals that are potentially very dangerous and require very special environments in captivity. They are highly social; if they cannot be permanently housed in social groups it is important that diverse social opportunities be devised.

Chimpanzees are used sparingly in behavioral research and as a surrogate for humans in some areas of study, such as human immunodeficiency virus (HIV) infection and viral hepatitis, where other species have not proven adequate. To develop a self-sustaining domestic supply of this important biomedical research resource, the National Institutes of Health has created colonies of behaviorally normal chimpanzees free of transmissible disease, to be used primarily for breeding. Some offspring will be made available for research.

Pan paniscus, the pygmy chimpanzee or bonobo, is found only in tropical rain forest areas of Zaire. They are not much smaller than *P. troglodytes,* but have a lighter build and a more rounded head. They are classified an endangered in all locations.

c. *GORILLA GORILLA*: GORILLA Three subspecies, discontinuously distributed, are known to exist: the western lowland gorilla (*G. gorilla gorilla*), mountain gorilla (*G. gorilla berin-*

gei), and eastern lowland gorilla (*G. gorilla graueri*). All are very large. Adult male weights are: *G. gorilla gorilla,* 140 kg; *G. gorilla beringei,* 155 kg; and *G. gorilla graueri,* 164 kg (Napier and Napier, 1985). Mature females weigh approximately half as much. Mature males are called "silverback" because of their saddle of gray hair across the back. Gorillas are an endangered species.

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CHAPTER 4

Functional Morphology

Jean E. Turnquist and Nancy Hong

NONHUMAN PRIMATES IN BIOMEDICAL RESEARCH: BIOLOGY AND MANAGEMENT

I. INTRODUCTION

Morphologically, nonhuman primates are more similar to humans (*Homo sapiens*) than to typical laboratory or domestic animal species. As with other orders, interspecific variations from the generalized norm can be characterized as an adaptation to a specific environmental niche and/or evolutionary divergence.

The general quadrupedal morphology of nonhuman primates can be divided into two patterns based on habitat use. Species that live primarily in trees have an arboreal-type morphology, whereas species that live primarily on the ground have a terrestrial-type morphology. Morphology classified by habitat use does not necessarily correlate with the groupings that are determined by evolutionary proximity.

Prosimians (suborder Prosimii, one of the suborders in the order Primates) are rarely used as laboratory animals and are not included in this chapter. Morphological details of how prosimians differ from other primates and are similar to other mammals are available in Martin (1990). In the United States, the Duke University Primate Center has had the most extensive experience with the animal husbandry of a variety of prosimian species.

The suborder Anthropoidea (higher primates) is divided morphologically into three groups based on evolutionary divergence. The first of these groups is the New World monkeys, infraorder Platyrrhini, that includes two families: Callitrichidae and Cebidae. The majority of New World monkeys in captivity are Cebidae; but, some facilities (for example, the Wisconsin Regional Primate Center) have had extensive experience with Callitrichidae. The second and third groups of Anthropoidea together comprise the infraorder Catarrhini. Although sometimes discussed as an infraorder, these two groups are more frequently referred to as the superfamilies Cercopithecoidea and Hominoidea. The Cercopithecoidea include all the Old World monkeys within the single family Cercopithecidae. The last group or Hominoidea includes three families: Hylobatidae (lesser apes), Pongidae (great apes), and Hominidae (humans). Each of these anthropoid groups has distinct morphological characteristics, yet many features are shared by all, including humans. Because of the morphological similarities between humans and nonhuman primates, books on human anatomy and/or surgery are usually excellent guides for nonhuman primates.

In addition to differing morphologically from most other laboratory or domestic animals, nonhuman primates are unusual in that they are generally multiple-use animals. They are used simultaneously and/or consecutively for a variety of purposes and are rarely euthanatized except when terminally ill. This long-term maintenance of nonhuman primates, including social housing and inhouse breeding programs, necessitates a depth of knowledge of their overall morphology beyond that which is necessary for most other laboratory animals.

Rhesus macaques (*Macaca mulatta*) have been the most common nonhuman primates housed in research facilities and thus most of the morphological descriptions in this chapter refer to this species. Major differences between macaques and other

species are noted whenever possible but interspecific variations are common. By providing a general description of primate anatomy, this chapter is intended to serve as a basis for the analysis and interpretation of the functional morphology of any of the higher nonhuman primates.

II. MORPHOLOGICAL DEFINITION OF PRIMATE ORDER

Morphologically, the order Primates is set apart from other orders primarily by its retention of many generalized features. Both fore- and hindlimbs have five digits and there is no reduction in the skeletal elements. For example, all primates have a complete shoulder girdle, including a well-developed clavicle and scapula, two distinct long bones in the distal segment of the limb, and no reduction in the number of bones in either the metacarpus or metatarsus. The grasping hands and feet (together called the cheirodia) which characterize the order have enhanced mobility of the digits, particularly the first on each of the four extremities. The digits usually end with a flattened distal phalanx which has a nail on the dorsum and a tactile pad on the palmar/plantar (volar) surface.

Cranial characteristics of primates in general include a short snout in conjunction with a poorly developed olfactory apparatus and a reduction in the number of teeth from the generalized mammalian number of 44. All four classes of teeth (incisors, canines, premolars, and molars) are present in primates, but their numbers may be reduced. The visual apparatus in primates is well-developed and the eye is protected by a complete bony orbit, including a complete postorbital bar. Vision is usually binocular with overlapping fields of vision. The cranial cavity is enlarged to accommodate the brain which shows considerable elaboration, particularly of the cerebral cortex. A posterior lobe and calcarine fissure are always present in the brains of Anthropoidea.

Throughout the order Primates the morphology shows a progression toward uprightness of the trunk, a trend which culminates in humans. All monkeys, apes, and humans have hemochorial placentas. The progressively later age of weaning and the lengthy social dependency between offspring and adults result in the prolongation of postnatal life periods characteristic of primates.

III. SEXUAL DIMORPHISM

The degree of sexual dimorphism within primates varies markedly among species. As a general rule, however, arboreal species usually have little sexual dimorphism, whereas sexual dimorphism in terrestrial species is usually more marked. The development of sexual dimorphism is primarily the result of different durations of growth periods in the two sexes, although rates of growth during a given time period or for a specific organ may also differ somewhat (Coelho, 1985; Gavan, 1985; Larson, 1985; Turnquist, 1984b; Turnquist and Kessler, 1989a).

Sexual dimorphism is evident in a variety of morphological characteristics. The most obvious of these is overall body size. Each species also has a variety of secondary sex characteristics such as hair color of the face and shoulders or the color of the perineum. In some species the intensity of the difference between the sexes varies greatly depending on the breeding cycle of the animals. Marked sexual dimorphism in body size is usually accompanied by marked differences in dental morphology, particularly canine size and the concurrent expansion of the maxilla and mandible. Thus males are generally more prognathic than females (Fig. 1 and 2).

IV. GROWTH AND DEVELOPMENT

A. Stages in Life Cycle

The life cycle of nonhuman primates can be divided into a series of stages, each one characterized by a distinct morphology and behavior. The names assigned to the stages and the criteria used to define each stage are not always consistent in the literature.

At birth, the neonate is well-developed, alert and active. The cranium is large in comparison to the body. Usually, the neonate is edentulous but occasionally deciduous incisors may be present. Both hands and feet are very well-developed and large in comparison to the limbs, and all four extremities are used to cling to the mother's hair. All primate neonates are dependent on their mothers at birth, but the intensity and duration of this dependency vary from species to species.

The infant is characterized morphologically by the eruption of the deciduous dentition and the concurrent development of the face. Behaviorally, the period is one of increased activity and exploration and ends at the time of weaning. The age of independence varies widely from species to species. Weaning does not occur until the motor skills of the infant develop sufficiently for it to negotiate the environment effectively. As a general rule, however, arboreal species tend to have a longer period of locomotor dependency. The rapidity, or slowness, of weaning in a given species parallels its overall rate of maturation. Species with short maturation times usually have short infancy periods while those with long maturation times usually have longer periods of marked dependency. The term weanling characterizes the level of development at the end of infancy.

The juvenile period spans the time between weaning and puberty. The juvenile locomotes independent of its mother and morphologically the most notable increases are in the growth of the limbs. During this time body proportions change rapidly and many of the distinguishing characteristics between New

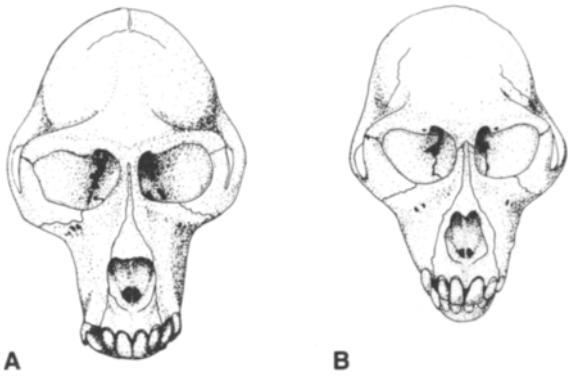

Fig. 1. Old World monkey skulls illustrating sexual dimorphism (superior-oblique view of *Macaca mulatta*, rhesus macaque). (A) Adult male; (B) adult female. Note differences in overall size, development of supraorbital ridges, breadth of zygomatic arches, length of face, and prognathism associated with differences in canine size and root development.

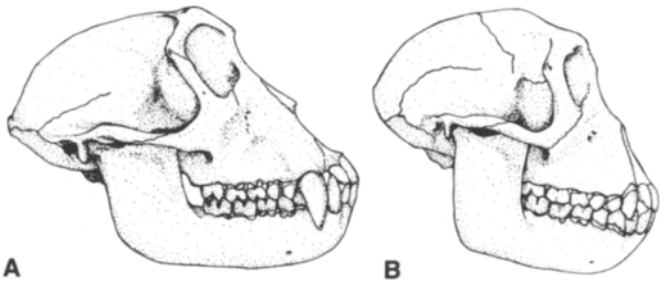

Fig. 2. Old World monkey skulls illustrating sexual dimorphism (lateral view of M. *mulatta*). (A) Adult male; (B) adult female. In addition to the features noted in Fig. 1, also note differences in size of the nuchal line (crest), width of the ascending ramus of the mandible, and length of the maxilla.

versus Old World monkeys and apes become evident. The first permanent teeth erupt and sexual dimorphism begins to become evident in those species where it is marked in adulthood. The adolescent growth spurt occurs late in the juvenile period which ends with puberty. In the literature, the juvenile period is sometimes subdivided into two or more levels of maturity.

Sexual maturity and morphological maturity, however, do not correspond and thus adulthood is subdivided into various phases with different morphological characteristics.

Young adulthood spans the time between sexual maturity and morphological maturity and usually lasts for several years. This stage is sometimes referred to as subadult or late juvenile in the literature. In most primates, sexual maturity occurs first, followed by dental maturity and skeletal maturity, and finally full adult body weight is achieved. New World primates, particularly the smaller species, can be exceptions to this sequence in that dental maturity is advanced and sexual maturity may be delayed. The pattern for skeletal maturation is that full adult length of the limbs is achieved before the full adult length of the body. The actual timing of the fusing of the various epiphyseal elements varies between the sexes and among the species, but the basic pattern of fusion remains fairly constant in all primates. Dental maturation, particularly the full eruption of the last (third) molar, usually occurs around the same time as skeletal maturity. The final component of the adult body physique to mature is the achievement of adult body weight.

Middle (or prime) age adulthood, the longest of the three phases of adulthood, is characterized by morphological stability with few, very gradual changes over the years. The term adult in the literature generally refers to this phase. Most of the changes which do occur during this period are cyclic in nature and are related to breeding/seasonal changes. Pathological conditions related to aging, e.g., arthritis, develop during this period but they do not noticeably affect the morphology or the behavior of the animal. Since the rate of development of some pathological conditions is influenced by environmental conditions, the actual chronological age of animals in their prime may vary even within a single species (DeRousseau *et al.*, 1986; Kessler *et al.*, 1986b).

Aged adulthood is the last of the phases in the life cycle. There is no specific criteria that separate prime from aged animals, but rather it is the cumulative effect of numerous morphological changes over the years. Systems typically affected by aging are the musculoskeletal, ophthalmological, neurological, and immunological systems. Most of the pathologies seen in nonhuman primates are those common to all mammalian species, including humans. In addition to pathological conditions per se, aged adulthood is also characterized by a general reduction in body size (i.e., wasting) and a decline in breeding success.

The basic morphological characteristics of the phases of the life cycle of primates are constant, but there is considerable variation between species, and even between sexes of the same species, on the actual duration of some of the phases. In addition to genetic factors, social and environmental factors contribute significantly to the morphology of nonhuman primates. In species with structured social orders, the morphology of an individual may be altered by rank due to access to food or other less tangible factors. Environmental conditions can also affect the morphology of individuals as evidenced in comparisons between free-ranging and laboratory animals (Kessler *et al.*, 1986b; Turnquist, 1983, 1984a, 1985; Turnquist and Kessler, 1990b). This effect is not necessarily only the result of nutritional differences, but also the result of differences in physical space and types of supports provided as well as social and psychological factors.

B. Dental and Skeletal Maturation

The sequence and age of dental eruption are not readily available for most primate species. The data available, however, show variations in the sequence of eruption in some teeth. In

general, for the higher primates, the first permanent teeth to erupt are the first molars and the last to erupt are either the premolars and canines or the third molars. The sequence of eruption of the second molars, the incisors, the third molars, and the premolars–canines complex varies among species. In most Old World monkeys and great and lesser apes, the second molars erupt after the incisors and the third molars after the premolars and canines. In *Colobus*, however, the second molars erupt after the central incisors and before the lateral incisors, but the premolars–canines–third molars sequence is the same as in other Old World monkeys. In New World monkeys, there is considerable variation in the sequence of eruption of the second molars and third molars relative to the other teeth (Cheverud, 1981; Fleagle, 1988; Schultz, 1933; Swindler, 1985; Turnquist and Kessler, 1990a,b).

The development of ossification centers in the extremities and the fusion of epiphyseal plates are methods of evaluating the age of immature animals (Michejda, 1987). Each species appears to have its own distinct timing for skeletal development, but actual data are not available for most species (Watts, 1990). Watts (1986) summarized the scant primate data available on the development of ossification centers both pre- and postnatally, and discussed the apparent major differences in timing and sequence. The same article also summarized all the available data on the age and sequence of epiphyseal fusion in primates. Since environmental conditions (laboratory vs free-ranging) affect both of these factors (Knezevich and DeRousseau, 1985; Turnquist, 1984a, 1985), published standards for even the most common primate (rhesus macaques) should be used with caution. Cheverud's (1981) evaluation of epiphyseal fusion in skeletal material of known age, free-ranging rhesus macaques from Cayo Santiago should be consulted when evaluating skeletal development.

V. BODY SIZE AND INTEGUMENT

A. Size and Sexual Dimorphism

Variability is one of the outstanding features of nonhuman primate morphology. The range in size among species is tremendous. For example, an adult male pygmy marmoset (*Cebuella pygmaea*) weighs less than 100 g whereas an adult male gorilla (*Gorilla gorilla*) may weigh more than 200 kg. These are two extremes of the range, but within the order there is considerable variability even between closely related species (Napier and Napier, 1967).

A second factor that affects body size is sexual dimorphism. The most marked differences between the sexes occur in species such as olive baboons (*Papio anubis*) and Sumatran orangutans (*Pongo pygmaeus abelii*) where adult female weight is approximately 50 and 54% of adult male weight, respectively. In other species, very little sexual dimorphism occurs such as in spider monkeys (*Ateles paniscus*) or chimpanzees (*Pan troglodytes*) in which adult female weight is approximately 103 and 90% of adult male weight, respectively. These comparisons of body weight differences are one index of the amount of sexual dimorphism in a species. The relationship of body weight to body length (crown–rump or head height plus trunk length) is such that those species in which the female weight is approximately 50% of the male weight, female body length is approximately 81% of that of the male. In species with little difference between body weight of males and females, there is also little difference between body length.

B. Skin

The skin of all primates is covered with hair. The thickness and density of the hair as well as its color and length vary significantly among species. The major determining criteria for differentiating species within a single genera are frequently coat (hair) color and pattern of coloration. Within a single species, however, coat color may show considerable variability, as evident in rhesus macaques in which coat color may range from brown to gray or even gold (Kessler *et al.*, 1986a; Napier and Napier, 1967). In addition to the basic coat coloration, many of the species also show considerable sexual variability in the coloration pattern of adults. For example, in patas monkeys (*Erythrocebus patas*), not only is the pattern of coat coloration different for males and females, but also the coloration of the facial hairs of the female changes during pregnancy and subsequent lactation (Palmer *et al.*, 1981).

Notable external coloration in primates is not just limited to hair. In some primates the skin on parts of the body, such as the face or perineum, assumes a coloration that is distinct for the species and/or phase of the reproductive cycle. For example, the skin of the face and perineum of adult male mandrills (*Mandrillus sphinx*) is multicolored with blue, white, and red, whereas the perineum of adult male patas monkeys displays a blue scrotum below a red perianal triangle flanked by the white hair of the posterior thighs. In many female primates the skin of the face and perineum shows marked reddening, sometimes accompanied by swelling, near ovulation and/or parturition. All of these colorations are thought to be integral parts of behavioral signaling within the context of the social organization.

The skin of nonhuman primates is histologically very similar to human skin. Both free (non-encapsulated) sensory epithelial and Meissner corpuscle (encapsulated) endings are present to transmit afferent impulses. As in humans, the hands and feet (Figs. 3 and 4) are covered by tactile pads complete with epidermal ridges on the volar surfaces (Cummins, 1933; Stewart, 1933). These pads have an abundance of afferent nerve endings which provide detailed sensory information to the brain (Niemitz, 1990). Some New World monkeys, such as spider (*Ateles*) or howling (*Alouatta*) monkeys, have prehensile tails which they use extensively as a fifth appendage. In these species, the

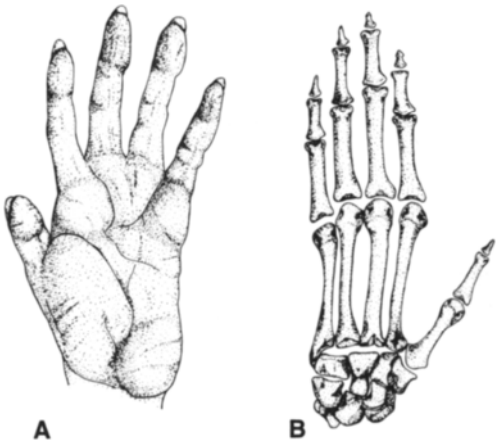

Fig. 3 Morphology of the left hand of *M. mulatta*. (A) Palmar surface; (B) dorsal view of the skeletal elements. Note the presence of a single palmar pad with creases and dermatoglyphics. The major creases reflect the functional divisions of the digits. The nails on the digits are located dorsally but are visible from the palmar (volar) view as they project beyond the digits. In the skeleton, note the separation between the first and second metacarpals. In the carpus, the pisiform lies anterior to the proximal carpal row and the lunate and os centrale are in very close approximation.

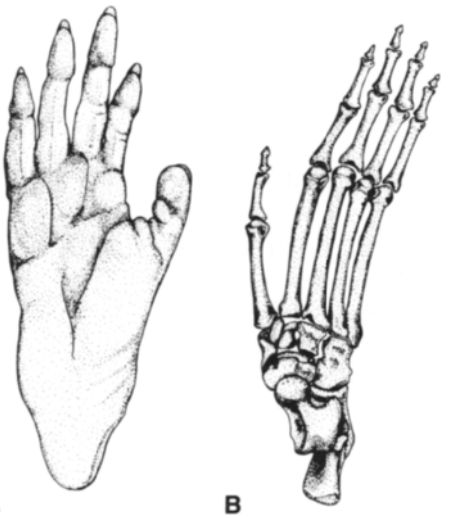

Fig. 4. Morphology of the right foot of *M. mulatta*. (A) Plantar surface; (B) dorsal view of the skeletal elements. Note the presence of a single plantar pad with creases and dermatoglyphics. As in the hand, the major creases reflect the functional divisions of the digits, the nails on the digits are visible from the plantar (volar) view, and, in the skeleton, there is a marked separation between the first and second metatarsals.

ventral surface of the distal tail is also covered by a tactile pad very similar to that on the hands and feet.

In Old World monkeys and gibbons (Hylobatid) the ischial tuberosities are covered by a callous formation: the ischial callosities. The form and extent of these callosities are species specific and in some species such as the Guinea baboon (*Papio papio*) the two fuse across the midline. There is, however, considerable variability within a single genus as evidenced by the well-separated ischial callosities of the rhesus monkey (*Macaca mulatta*) and the fused callosities of the Barbary macaque (*Macaca sylvanus*). The extent of these callosities is thought to be related to sitting habits, but the correlation between behavior and morphology has not been proven in controlled studies.

The only part of the primate body with extensive voluntary muscular control over the skin is the head and neck region with its emphasis on the muscles of facial expression, mimetic musculature. The wide range of facial expressions found among nonhuman primates has been extensively studied for its role in social communication. Marked similarities between some facial expressions in all species of primates exist and signals made by one species (including humans) can be clearly interpreted by another. Voluntary skin mobility of parts of the body other than the head and neck is very limited in nonhuman primates with the exception of the insertion of a few muscle fibers into the skin over the lower thoracic and abdominal regions (m. panniculus carnosus).

C. Nails or Claws

All Cebidae, Cercopithecoidea, and Hominoidea have nails on the dorsum of the distal phalanges of all 20 digits on the hands and feet. The structure of these nails and the shape of the distal phalanges in nonhuman primates are similar to that described for humans. The Callitrichidae differ from this by having nails only on the halluces. The claws on the other digits, however, are more nail-like than a typical mammalian claw and the underlying distal phalanges are only moderately claw-shaped.

VI. HEAD AND NECK MORPHOLOGY

A. Superficial Musculature

The muscles of facial expression, mimetic musculature, are prominent features of the head and neck morphology of nonhuman primates (Huber, 1933). The variety of movements of the skin of the eyelids, lips, ears, neck, and nostrils is critical for interanimal signaling within the context of the social organization. Visual displays, as well as vocal repertoires, are essential to communicate everything from threats and aggression to unease or sexual solicitation.

B. Ear

The external ears of primates are positioned laterally on the skull. The pinna varies in size from one species to another but is generally small relative to head size. There is interspecific variability in the shape of the external ear but usually it is oval with some infoldings (Bast, 1933). The pinna is generally im-

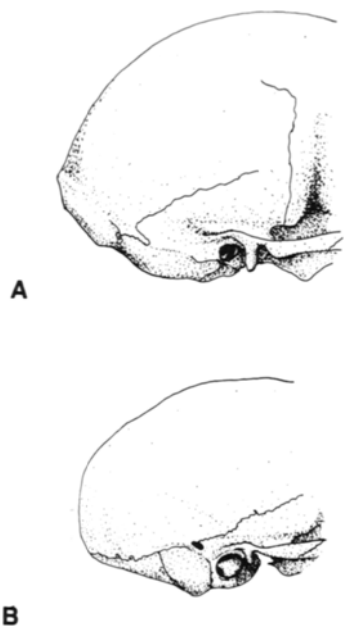

Fig. 5. External ear configuration in Old World versus New World monkeys. (A) Old World monkey (*M. mulatta*); (B) New World monkey (*C. apella*). Note the lateral bony extension in the Old World monkey forming an external auditory tube. In the New World monkey the tympanic ring for the tympanic membrane is on the surface of the skull and the ear ossicles are clearly visible. In both the temporomandibular articulation is immediately anterior to the opening.

mobile and Old World monkeys have muscles for only retraction of the ears. The external ear is covered by hair which varies in density between species and may form an ear tuff on the superior point of the pinna.

The external auditory tube lies between the external opening of the ear and the tympanic ring and eardrum. The structure of this tube varies along evolutionary lines. In Old World monkeys and apes the bony tympanic ring is prolongated laterally so the medial portion of the external auditory tube is bone rather than cartilage. New World monkeys lack this prolongation so the tympanic ring is exposed on the side of the skull and the entire meatus is cartilagenous (Fig. 5).

The middle ear of all primates is bordered laterally by the tympanic ring which encircles the eardrum. The osseous floor is formed by the petrous portion of the temporal bone. In New World monkeys this floor is ballooned superiorly to form the tympanic bulla. Old World monkeys and apes lack this ballooning of the floor.

The inner ear occupies hollowed out canals within the petrous portion of the temporal bone immediately adjacent to bony tubes housing the internal carotid artery and its branches.

C. Eye and Orbit

The eyes of primates are distinct from most other animals in that they have binocular vision. The orbits are relatively close together and oriented anteriorly which results in overlapping of their visual fields (Bast, 1933). The bony orbit is complete with a well-developed postorbital bar and a postorbital plate which separates the orbit from the infratemporal fossa. The size of both the eye and orbit varies among species but the relative size coincides with the behavioral habits of the species. In the owl monkey (*Aotus*), a nocturnal species, the eyes and orbits are relatively large compared to the size of the skull. In diurnal species the orbits and eyes are relatively small.

Many morphological similarities exist between the eyes of nonhuman primates and humans. The primate retina contains both rods and cones for reception of visual stimuli. All higher primates lack a tapetum lucidum. The centralized macula has an abundance of cones and visual acuity is sharpest around the fovea centralis. Among monkeys and apes the only known exception to this pattern of retinal morphology is the nocturnal owl monkey (*Aotus*) which lacks cones in its retina and whose fovea centralis contains only rods. The owl monkey is also the only higher primate to lack a macula lutea.

As in humans, the lacrimal gland is located in the superolateral portion of the orbit and the duct inferiomedially. The secretions of this gland constantly bathe the cornea. Like humans, nonhuman primates develop age-related pathologies of the eye such as cataracts, macular degeneration, and loss of visual acuity. The superior and inferior eyelids are single extensions of the skin. In some species the superior lid may have distinctive coloration. Raising, or lowering, the brows are important components of the communicative behavioral repertoire of primates. They can expose, or conceal, the upper eyelids and widen, or narrow, the eye slit to display, or obscure, the eyeball.

D. Nose

The external morphology of the nasal region in nonhuman primates is characterized by the lack of a rhinarium, lack of a primitive type philitrum, and an upper lip which is continuous inferior to the nasal septum. The size and shape of the nasal bones and cartilagenous elements vary considerably between species and/or sexes. Two basic patterns for primate nasal morphology are evident and divide along evolutionary lines. The nostrils of most New World monkeys are widely placed and forward facing, hence the name platyrrhine. The nostrils of most Old World monkeys and apes are closer together, outward facing, and are separated by a narrow septum. Hence, these groups are called catarrhines.

Concomitant with the closer approximation of the orbits in all higher primates, the cribiform plate is displaced vertically and the ethmoturbinals are arranged more vertically than horizontally. The olfactory nerves (cranial nerves I) have a limited distribution in the upper portion of the nasal cavity and exit the region superiorly (Geist, 1933). In primates, olfaction is not highly developed when compared to many other mammalian groups. Despite this underdevelopment, some nonhuman

primates, particularly New World monkeys, exhibit scent-marking behavior using either secretions from perineal or sternal glands or urine. Adult platyrrhines have apparently functional vomernasal (Jacobson's) organs for chemical sensing whereas those of adult catarrhines are only vestigial (Martin, 1990).

E. Lips

The mouth of all primates is characterized by continuous upper and lower lips which in some species are highly mobile and frequently used in social displays. Opening of the mouth to display the teeth is common in some types of social encounters.

F. Dentition

The upper dental arch is formed by the alveolar bone of the premaxilla and maxilla of the skull. The lower dental arch is formed by the two halves of the mandible which in higher primates are always fused on the midline. The shape of the dental arches ranges from rectangular to semicircular or U-shaped depending on the species and/or sex of the animal. Dentition is usually described by quadrants of the mouth: right and left maxillary (upper) and right and left mandibular (lower) quadrants. Four classes of teeth (Butler, 1978) are evident in each quadrant of higher primates (Fig. 6). From anterior (midline) to posterior these are incisor, canine, premolar, and molar (James, 1960; Marshall, 1933).

The nomenclature used to describe teeth in higher primates is not always consistent from one reference to another. The nomenclature used here is one of the most commonly used for humans. It has also traditionally been used in primate literature on dental morphology, development, and evolution. It is based on quadrants and identifies each tooth individually as to class (denoted by a single letter) and order of its location for that class (denoted by a number). Incisors are abbreviated I and are numbered from the midline. I1 is the central incisor and I2 is the lateral incisor. The canine tooth is abbreviated C and since there is only one canine in each quadrant it is written C1.

Premolars are abbreviated P although Pm or PM have occasionally been used in the literature. The numbering system for premolars is based on studies of the evolutionary reduction in the number of premolars from the primitive mammalian number of four. These studies have shown that premolars are lost from anterior to posterior along the tooth row and that the premolars retained in living primates are the last of the original premolars. Thus, the premolars present in living primates are P2, P3, and P4 if the primate has three premolars and P3 and P4 if the primate has only two premolars. Use of this system allows an accurate description and comparison of a specific tooth regardless of its evolutionary or developmental stage or the number of premolars present in the species. This type of designation, however, has not been used consistently by researchers and clinicians who are unfamiliar with the evolutionary history of the premolars. Some literature may therefore incorrectly refer to premolars as P1, P2, and P3 when they should accurately be described as P2, P3, and P4. The last tooth class in each quadrant is the molar which is abbreviated M. Individual molars are designated as M1, M2, and M3.

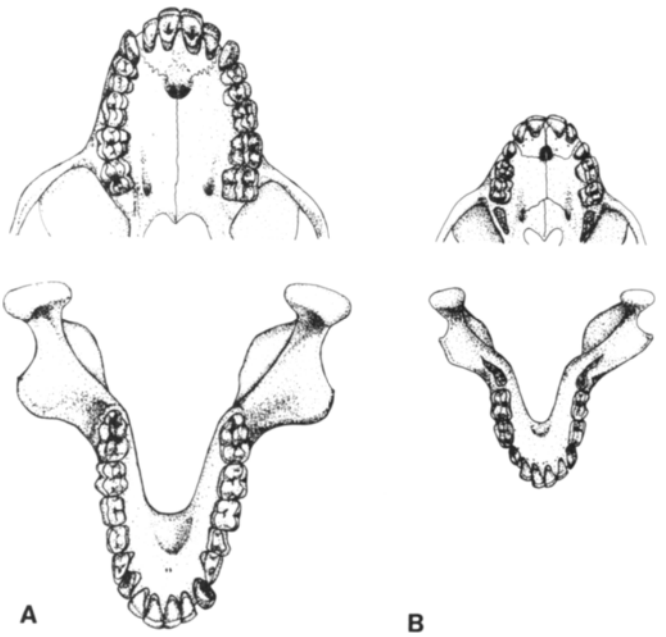

Fig. 6. Old World monkey dentition (*M. mulatta*, female). (A) Permanent dentition (adult); (B) deciduous dentition (juvenile, age 1.0 year). In the adult, note the number of premolars in each quadrant, the diastema between the lateral incisor and the canine, and the cusp pattern of the molars. In the juvenile, note the number and cusp pattern of the premolars (called milk "molars"), the presence of the first permanent molar in its crypt in each of the four quadrants, and the relative size of the deciduous teeth as compared to the permanent teeth in the adult.

In addition to each tooth in the tooth row being designated by a letter and number, the position of the number for a given tooth is used to indicate the dental arch in which the tooth appears. If the number is written as a superscript, the tooth is in the maxillary dental arch. If the number is a subscript, the tooth is in the mandibular dental arch.

Like humans, nonhuman primates have two distinct sets of dentition (Fig. 6). The first of these, the deciduous or milk dentition, occupies the same position in the dental arch as the subsequent successor teeth. The letter d before the tooth notation is commonly used in the literature to indicate a deciduous tooth, although deciduous teeth are sometimes indicated in the lower typecase (i, c, p, or m) instead of the capital typecase (I, C, P, or M) used to indicate permanent teeth. Typically, the deciduous teeth of each dental quadrant include two incisors, dI1 and dI2; one canine, dC1; and two premolars, dP3 and dP4, in Old World monkeys and apes or three premolars, dP2, dP3, and dP4, in New World monkeys. As indicated here the deciduous cheek teeth are premolars even though they are frequently called "mo-

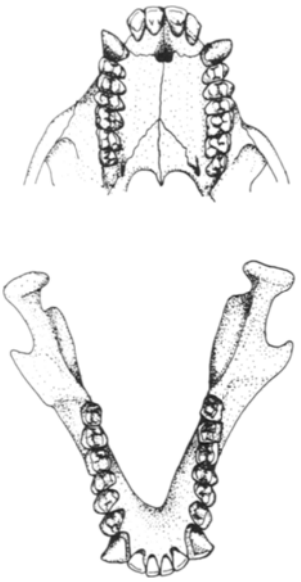

Fig. 7. New World monkey permanent dentition (*C. apella*, female). Note the number of premolars in each quadrant, the relative size and shape of the premolars versus molars, and the shape of the incisors.

lars." The sequence and age of dental eruption are discussed with growth and development (Section IV,B).

Deciduous teeth are generally smaller than their successor teeth. Anterior deciduous teeth have many of the same characteristics as their permanent counterparts. Deciduous cheek teeth, on the other hand, more closely resemble permanent molars rather than the premolars which succeed them. This has resulted in clinicians traditionally identifying these premolars as molars. The total number of deciduous teeth is 20 in Old World monkeys and apes and 24 in New World monkeys. As in humans, the first permanent molars in nonhuman primates usually erupt before the deciduous teeth are replaced by the permanent successor teeth.

The permanent dentition of higher primates is usually complete soon after skeletal maturation is achieved. In some species, particularly the smaller New World primates, dental maturity may be accelerated and may precede skeletal maturity. The dental formula (James, 1960) for Old World monkeys and apes is written as 2.1.2.3./2.1.2.3. for a total of 32 teeth (Fig. 6). This formula indicates two incisors, one canine, two premolars, and three molars in an upper quadrant and a similar pattern in a lower quadrant. The dental formula for New World monkeys other than Callitrichidae is written as 2.1.3.3./2.1.3.3. for a total of 36 teeth (Fig. 7). The dental formula for Callitrichidae is usually 2.1.3.2.1./2.1.3.2. for a total of 32 teeth but only two molars per quadrant.

The two most anterior teeth in each quadrant are I1 and I2. The crown of each incisor is generally broad and spaculate. The single root is usually conical. The roots of upper incisors are usually rounder and longer than those of lower incisors. The upper incisors are the only teeth rooted in the premaxillary bone. The incisors of primates play an important role in food acquisition.

Posterior to the incisors and the premaxillary suture is C1. Both the crown and the root of the canine are conical. The size and shape of the tooth vary greatly among primate species, between the sexes, and between the maxillary and mandibular dental arches. The canine usually projects beyond the regular tooth row and a diastema is frequently evident between the upper lateral incisor and the canine. In occlusion the lower canine usually fits into the diastema and thus lies anterior to the upper canine (Fig. 2). As the jaw closes the posterior border of the upper canine slides against the adjacent lower cheek tooth. The posterior aspect of the upper canine can be honed to a razor sharp edge in some species where the canine projects well beyond the tooth row. In terrestrial species with marked sexual dimorphism the upper canine of the adult male may project as much as seven times the height of the rest of the dental row. In other species, such as some arboreal species with little sexual dimorphism, the canines of both sexes may project only slightly beyond the rest of the dental row. The very large upper canine seen in some primates is generally considered to have a social function instead of a food acquisitional function.

The cheek teeth of primates are used for mastication and are divided into two groups based on evolutionary development as well as dental morphology. The teeth posterior to C1 are the premolars. In the bicuspid premolars of higher primates, the lateral or outer or buccal cusp is normally larger. The only exception to the usual bicuspid premolars in higher primates is the lower P3 in Old World monkeys and great apes. In this exception P3 is unicuspid and the anterior surface forms a sloping shoulder which can have a shearing interaction with the upper C1. Upper premolars generally have three roots whereas lower premolars have two (see Remane, 1960, for a detailed count of roots of teeth in various species). The range of species variability in premolars is greater than in other teeth.

Unlike the other teeth, the number of premolars in each quadrant is not constant for all higher primates but divides into two distinct patterns along evolutionary lines. New World monkeys have retained three, i.e., P2, P3, and P4, of the original primitive mammalian number. Old World monkeys and apes (as well as humans) have retained only two premolars, i.e., P3 and P4. This distinction between the two evolutionary groups is evident in both the deciduous and permanent dentition.

The last teeth in the dental arch are the three molars: M1, M2, and M3. As in premolars, upper molars generally have three roots whereas lower molars generally have only two. All molars typically have four to five cusps although the number may be reduced, or expanded, especially in the last tooth of the row. Reduction or even absence of the third molar is more common in, but not limited to, New World monkeys. The Callitrichidae are the only higher primates that consistently have only two molars per quadrant. Expansion of the third molar is most common in the longer faced species of Old World

monkeys. There is, however, considerable variability even between closely related species. In Old World monkeys, the molar crowns display a narrowing between the anterior and posterior cusps, resulting in two distinct ridges which are oriented lingual–buccally and the resultant molar is called bilophodont.

G. Oral Cavity

Within the oral cavity the tongue plays an important role in maintaining food between the grinding surfaces of the teeth. The tongue of primates is relatively short and flat and lacks the food-acquiring mobility of some other animals. As in humans, the tongue surface of nonhuman primates has an abundance of papillae with numerous taste buds and their associated sensory nerve endings. The tongue is divided into an anterior three-quarters and a posterior one-quarter by a V-shaped row of circumvallate papilla. The anterior (ventral) three-quarters of the tongue has numerous fungiform papillae particularly near the tip. Smaller filiform papillae give the entire tongue a relatively smooth appearance. On the posterior one-quarter of the tongue foliate papillae lie adjacent to the lingual tonsils. The pharynx is relatively short and straight and the line of the passage from the oral cavity through the pharynx and into the larynx is nearly straight (Geist, 1933).

Three pairs of salivary glands provide secretions to the mouth. Each parotid gland is located caudal and ventral to the external auditory meatus. The gland lies superficial to the internal carotid artery, the internal jugular vein, and numerous nerves. It is traversed by the facial nerve (cranial nerve VII), the external carotid artery, the external jugular vein, and other smaller nerves and vessels. The parotid duct enters the oral cavity lateral to the upper M1. The submandibular (submaxillary) gland lies inferior and medial to the angle of the mandible and is in close proximity to the hypoglossal nerve (cranial nerve XII) and the major vessels in the neck. The submandibular duct enters the floor of the mouth inferior to the tongue. The sublingual gland is the smallest of the three and lies immediately deep to the oral mucosa. It lacks a single duct and instead has numerous openings directly into the floor of the mouth.

The mouths of many, but not all, Old World monkeys have an additional unique characteristic in that they have cheek (buccal) pouches which are extensions of the oral cavity. These superficial pouches extend for a considerable distance into the neck on either side of the midline. Each pouch is covered by muscle fibers derived from the m. buccinator which have herniated through the overlying m. platysma. The mucous membrane lining the pouch is continuous with that of the rest of the buccal surface of the mouth. Cheek pouches are frequently used to store food which will later be pushed back into the oral cavity (sometimes by hands placed external to the pouch) for further mastication prior to swallowing. The food in cheek pouches is not regurgitated material, but food stored prior to mastication.

H. Deep Musculature

The deep musculature of the head and neck region is divided into groups according to function. The first of these are the muscles of mastication. The four pairs of muscles in this group are mm. temporalis, mm. masseter, mm. medial pterygoid, and mm. lateral pterygoid. Use of the first three of these pairs in a single coordinated action closes the jaws. The last pair, mm. lateral pterygoid, assists neck muscles in opening the jaw. Various combinations of these muscles also result in protraction, retraction, and side-to-side movement of the jaw.

The anterior neck musculature in higher primates is subdivided according to its position relative to the visceral package. [The visceral package encloses the pharynx and its two inferior (caudal) extensions, the trachea and esophagus. The contents of the visceral package are discussed in later sections: the trachea and larynx with the respiratory system (Section IX,G) and the pharynx and esophagus with the gastrointestinal system (Section X,D).] The muscles superficial to the visceral package are the supra- and infrahyoid muscles. This series of strap-like muscles attach the hyoid bone to the mandible superiorly and the trachea, sternum, clavicle, and ribs inferiorly. All play an active role in movements of the hyoid bone, particularly during swallowing.

Lateral to the visceral package in the neck lies the complex of muscles which in humans is called the m. sternocleidomastoideus and the deeper mm. scalenii. As a group these muscles act either to flex the neck or elevate the rib cage, depending on which end of the muscles is fixed. When used ipsilaterally they can laterally bend the neck. By combining muscles with similar fiber directions, various combinations of muscles can also rotate the neck and head. Posterior to the visceral package, the prevertebral muscles attach to the bodies, pedicles, and transverse processes of the cervical vertebrae and the basioccipital of the skull. Functioning as a unit they flex the neck and bend the head forward.

The posterior or nuchal muscles of the neck include superficial muscles, many of which migrated secondarily into the region as well as deep muscles which are intrinsic to the region. The m. trapezius has a broad attachment on the occipital bone and in many species of higher primates this attachment is raised into a distinctive nuchal crest. The size of the nuchal crest (Fig. 2) varies greatly among species and between sexes. The extent of its development is correlated with the need to posturally balance the weight of the anterior part of the skull and face. The relatively short neck, broad chest, and concurrent dorsal placement of the scapula seen in most primates results in the superficial neck muscles having a slightly different orientation than that commonly seen in quadrupeds. The intrinsic muscles of the

posterior neck are continuous with, and a part of, the longitudinal musculature which extends from the occipital of the skull to the distal tip of the tail.

I. Skeleton

The skull of higher primates differs from that of other animals in several features (Figs. 1 and 2). First, the eyes are completely surrounded by bony orbits. Lateral to each eye, a complete postorbital bar is connected to the braincase by a bony postorbital septa which completely divides the orbit from the temporal region. This change in the orbit results in the dissociation of the rostral part of the zygomatic arch from the inferior aspect of the orbit and makes the infratemporal fossa a discrete area. The cranial vault is expanded to allow for the expansion of the brain which is found in higher primates. The external surface of the vault may be marked superiorly by a sagittal crest delineating the borders of well-developed mm. temporalis or posteriorly by a nuchal crest marking the attachment of the mm. trapezius. These two crests may appear separately or together and may differ greatly in size among closely related species and between sexes. The degree of development of the sagittal crest is thought to be related to the forces of mastication, whereas the development of the nuchal crest reflects the need to posturally balance a heavier face and anterior skull.

The overall size and shape of the primate skull differ among species and between sexes. The skulls of Old World (Figs. 1 and 2) and New World (Fig. 8) monkeys differ in ear, nasal, and dental arch morphology as well as in the degree of prognathism and sexual dimorphism. Palate length in most species is relatively short when compared to skull length.

The position of the foramen magnum and the occipital condyles that lie lateral to it varies from species to species, but in all primates they are primarily inferior rather than posterior. The flexure of the basocranium associated with this positioning permits a more vertical posture of the trunk relative to the skull.

The temporomandibular joint lies immediately anterior to the external acoustic meatus. The mandibular condyle is well-developed and longer mediolaterally than anteroposteriorly. The angle of the ascending ramus of the mandible varies among species and/or sexes, but the temporomandibular joint is always positioned superior to the tooth rows. The coronoid process is well-developed since m. temporalis plays a major role in primate mastication. The mandible of higher primates is fused on the midline and acts as a single unit in articulation with the skull.

The seven cervical vertebrae of primates include the commonly seen mammalian variations in the form of the atlas, C1, and the axis, C2 (Figs. 9A–9C). The body of a typical cervical vertebra is saddle-shaped, broad, and relatively thin. Foramen in the laterally flaring transverse processes of a typical cervical vertebra transmit the vertebral arteries between the thoracic inlet and the cranium. In higher primates the posterior (dorsal) arch of the atlas not only lacks a spine but also is very narrow (Fig. 10). The spines of the axis and other cervical vertebrae are narrow craniocaudally when compared to those of many other mammals (see also Section VII,C).

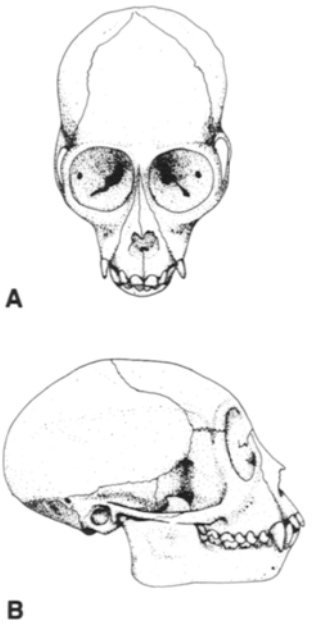

Fig. 8. New World monkey skull (*Cebus apella*, adult female). (A) Superior-oblique view; (B) lateral view. Note the relative width of the parietal eminences and zygomatic arches, lack of development of supraorbital ridges, a nearly vertical facial profile associated with little prognathism, and a wide ascending ramus of the mandible. Sexual dimorphism of the skull is not marked among most New World monkeys.

J. Brain

The primate brain is relatively large and incorporates more complex structural modifications than the brain of most other animals of similar body size (Hines, 1933; Noback and Montagra, 1970). The major lobes of the cerebral hemispheres (temporal, frontal, and occipital) are all well-developed. The frontal lobes overlay the reduced olfactory bulbs and the occipital lobes overlay the cerebellum. Major sulci present in all higher primates are the rhinal, Sylvian, and calcarine sulci. The sulcal pattern of the cerebral cortex in higher primates varies from species to species. The sulcal pattern of Callitrichidae is relatively simple compared to the very complex patterns found in Cebidae, Cercopithecidae, and Hominoidea. Noback and Moskowitz (1963) described species variations in the brain and spinal cord of higher primates and correlated these with primary motor and sensory projection areas. In all higher primates, the brain receives blood from both the vertebral and internal carotid

arteries. The promontory branch of the latter artery lies adjacent to the middle ear and is usually the major source of blood to the cerebral cortex.

K. Neurovascular Systems of Head and Neck

The innervation to structures in the head and neck region of nonhuman primates in general is the same as in humans (Christensen, 1933; Howell and Straus, 1933b; Kuntz, 1933). The cervical spinal nerves and the 12 cranial nerves which include both somatic and autonomic (parasympathetic) fibers as well as the ganglia and postganglionic fibers of the sympathetic nerves follow the basic patterns described in books on human anatomy.

The vascular system in the head and neck of nonhuman primates, including arteries, veins, and lymphatics, follows the basic patterns described in books on human anatomy.

L. Clinically Significant Features of Head and Neck Morphology

Clinically significant features of head and neck morphology include the approaches for obtaining samples of blood and cerebrospinal fluid and the use of dental eruption for aging of immature animals (see Section IV,B).

A common site for venopuncture in some animal species is the external jugular vein. Although this vessel is accessible in higher primates, it is usually easier to obtain blood samples from either the femoral or saphenous veins. The external jugular vein in higher primates is relatively short and drains blood almost exclusively from the region of the face. The brain and deep face are drained by the internal jugular vein which joins the external jugular vein near the root of the neck. In Old World monkeys with cheek pouches the exploration of the extent of the recesses should precede venopuncture of the jugular vein.

Samples of cerebrospinal fluid from higher primates are readily obtainable by entering the subarchnoid space either between the base of the skull and the first cervical vertebra or between the first and second cervical vertebrae (C1 and C2). Marked flexion of the head and neck opens up spaces between the posterior (dorsal) arch of C1 and both of the adjacent bones. The nuchal ridge of the skull and the spine of C2 are easily palpable bony landmarks (Fig. 10). Withdrawal of cerebrospinal fluid lower in the vertebral column is hampered by the broad placement of the articular processes of the lumbar vertebrae and the cranial placement of the iliac blades of the pelvis.

VII. BACK AND SPINE MORPHOLOGY

A. Overview of Back and Tail

The vertebral column of higher primates typically has 26–31 vertebrae, exclusive of the tail, with most variability occurring in the lumbar and sacral regions. The functional components of the back, however, are more important than the actual number of vertebrae in any given region. Differences in the functional lengths of various regions are of particular importance in the lumbar region and can be correlated with the most common locomotor patterns of the species (Erikson, 1963).

Externally, the tips of the vertebral spines are palpable along the midline of the back. The relative mediolateral flattening of the thorax, with the concurrent more posterior positioning of the scapulae and the flaring of the ilia both cranially and posteriorly, results in the intrinsic back region forming a relatively narrow strip on either side of the midline. Specializations in the cervical region have been discussed in Section VI,I on head and neck morphology.

The caudal region of higher primates shows considerable variability, particularly in external morphology. Some primates such as the great and lesser apes, as well as humans, lack evidence of an external tail. All New World and Old World monkeys have tails but these vary in length from short with little dexterity to long with tremendous dexterity.

The tails of all New World monkeys are relatively long and those of two subfamilies, the Atelinae and the Alouattinae, are prehensile and have a tactile pad on the ventral surface (German, 1982). This pad is very similar to the palmar/plantar pads on the hands and feet and in these species the tail is frequently used as a fifth extremity. Other families also have some functional prehensibility in their tails but lack the pressure pads necessary for true prehensibility.

Among Old World monkeys there is tremendous variability in tail length even among closely related species. No Old World monkey has a prehensile tail even though in some species young animals may occasionally wrap their tails around supports and use their tails for balancing.

B. Musculature

Internally the muscles of the back can be subdivided into extrinsic and intrinsic groups with the latter group including the muscles of the tail (Howell and Straus, 1933a). The extrinsic muscles include hypaxial muscles of the pectoral and pelvic girdles which have migrated to the posterior midline and attached to the spines and transverse processes of the vertebrae. In primates the relatively short neck and posterior position of the scapula result in the extent and positioning of the pectoral girdle muscles, particularly mm. trapezius and latissimus dorsi, being generally broader in origin and shorter in length than in most other quadrupeds. All of these hypaxial muscles are innervated by ventral rami of spinal nerves.

The intrinsic muscles of the back extend from the skull to the tip of the tail. In primates, these muscles are similar to epaxial muscles in most other mammals. Each muscle bundle extends for a limited number of segments and overlaps with adjacent bundles. Muscle fibers become progressively shorter in more

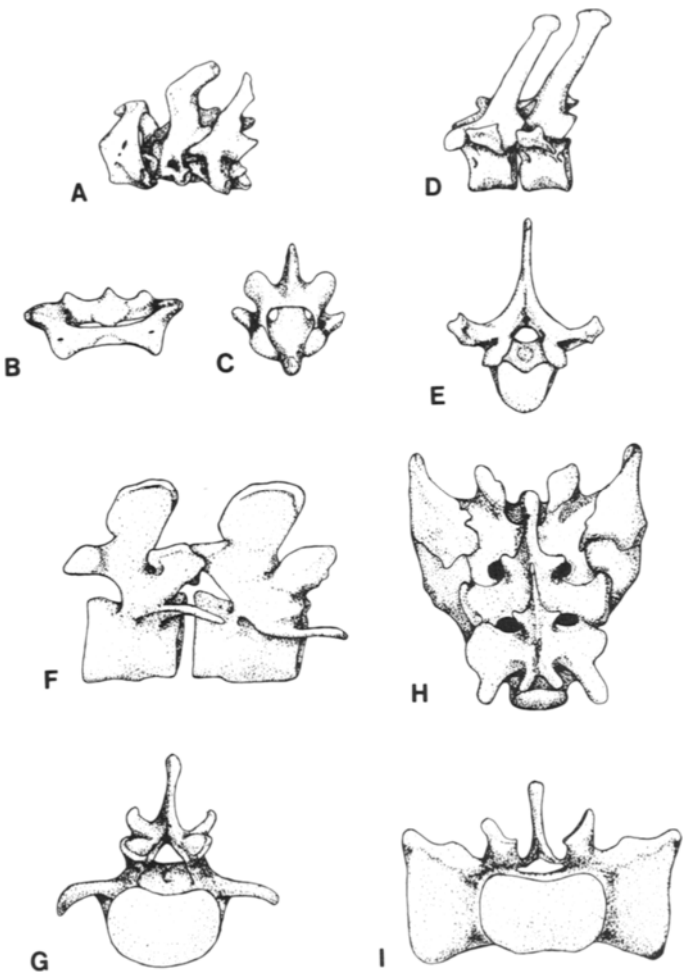

Fig. 9. Regional differences between vertebrae (*M. mulatta*). (A–C) Cervical vertebrae. (A) Lateral view C1 through C3, (B) posterior view C1, (C) superior view C2. (D and E) Thoracic vertebrae. (D) Lateral view T5 and T6. (E) superior view T6. (F and G) Lumbar vertebrae. (F) Lateral view L4 and L5, (G) superior view L5. (H and I) Sacrum. (H) Dorsal (posterior) view, (I) superior (cranial) view S1. Note that the important characteristics of each region are shown here but that the vertebral column is a continuum and individual vertebra vary depending on their relative position. The sizes and shapes of the spines, articular facets, and bodies of one region blend with those of the adjacent region.

internally placed muscles. As in other mammals, epaxial muscles are innervated by dorsal rami of spinal nerves.

The musculature of the tail is a direct continuation of the intrinsic muscles in the rest of the vertebral column. In primates lacking external tails these muscles, like the caudal vertebrae, are poorly developed. In other primates these muscles are developed in direct relationship to the length and dexterity of the tail. Among prehensile-tailed New World monkeys these muscles are highly developed. The majority of the ventral and lateral musculature of the tail is innervated by ventral rami of spinal nerves while dorsal rami contribute to the innervation of the dorsal and lateral musculature.

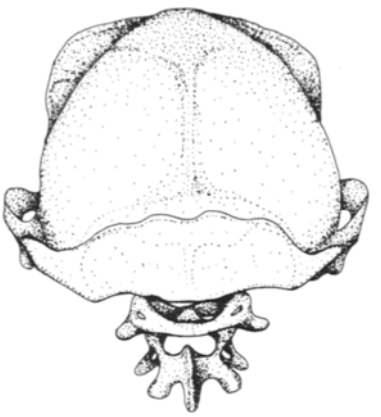

Fig. 10. Skeletal components of the nuchal region with the head flexed ventrally (dorsal view of the skull and the first and second cervical vertebrae of an Old World monkey, *M. mulatta*). Note the thin dorsal (posterior) arch of the first cervical vertebra and the short spine of the second cervical vertebra. Flexion of the head increases the spaces between the occipital bone of the skull and C1 as well as the space between C1 and C2.

C. Skeleton

The vertebral column is divided into five distinct anatomical and functional regions. Typically, primates have seven cervical vertebrae (C1 to C7). Details of clinically important modifications of C1 and C2 (Figs. 9A–9C and 10) are described in the section on head and neck morphology (last paragraph in Section VI,I). The first six cervical vertebrae typically have foramen transversarium and usually the vertebral artery enters the resultant canal at the level of C6. The transverse processes of C7 are generally long and slender. The articulations between the skull and C1 allow for movements of the head, which result in moving of the head as if indicating "yes." The articulations between C1 and C2 allow for rotation or movement of the head as if indicating "no." Movements between the remaining cervical vertebrae are primarily flexion and extension, although lateral bending is also possible due to the thickness of the intervertebral discs.

Most primates have 12 thoracic vertebrae (T1 and T12), although some individuals may have as many as 13 or as few as 10. All thoracic vertebrae provide articulations for a pair of ribs (Figs. 9D and 9E). The rib of the same number generally articulates with both the superior (cranial) part of the body and the transverse process of the thoracic vertebrae with the same number. In addition to these articulations, the upper thoracic vertebrae (generally T2 through T9) also have an inferior (caudal) articulation on the body for contact with the rib of the adjacent vertebrae. Spinous processes of thoracic vertebrae are usually long and narrow and overlap the spine of the adjacent more caudal vertebra. Most rotation of the vertebral column occurs in the thoracic region.

The lumbar vertebrae (L1 to L7) constitute the region of the vertebral column in higher primates with the most variability in number. Some species usually have as few as four lumbar vertebrae while others usually have seven (Figs. 9F and 9G). In general, all lumbar vertebrae have large bodies and large broad spinous processes. Their transverse processes become progressively longer and more massive from cranial to caudal. An exception is the last lumbar vertebrae which may be smaller and in close proximity to the adjacent borders of the ilia.

The number of lumbar vertebrae not only varies greatly among species but also there is considerable variability within a single species. The most common number for Old World monkeys is seven, for lesser apes five, and for great apes four, whereas New World monkeys range from four in *Ateles* and *Lagothrix* to six or seven in *Cebus*. The number of actual vertebrae in this region can be roughly, but not precisely, correlated with the locomotor behavior or functional role of the region. Erikson (1963) demonstrated this correlation between the use of the region in common patterns of locomotion and its functional length. Although not a perfect fit, his analysis does provide evidence of functional differences associated with morphological variability in the region. Most of the flexion and extension as well as a large amount of the lateral bending of the vertebral column occur in the lumbar region.

The sacral vertebrae (S1 to S5) of higher primates are fused after infancy (Figs. 9H and 9I). The sacrum of great and lesser apes generally are the result of fusion of four or five sacral vertebrae, whereas those of most Old World and New World monkeys generally incorporate only three sacral elements. The ala of the sacrum are broad and offer an extensive articulation with the ilia. The first sacral segment is always the largest with the size decreasing in each adjacent, more distal segment. The size of the last sacral segment is in part correlated with the length of the tail and the volume of the spinal segment nerves it transmits.

The caudal vertebrae vary tremendously in form and number among the various species of primates. In all cases, however, they become progressively smaller in diameter and more modified in morphology (German, 1982). Typical vertebral characteristics that are lost in an orderly fashion from proximal to distal along the caudal vertebrae are spine, vertebral foramen, articular processes, and transverse processes. The articulations between adjacent vertebral bodies become rounded and chevron bones are associated with the more proximal elements. In caudal vertebrae the height (cranial–caudal dimension) of the vertebral body becomes much greater than the width (anterior–posterior or medial–lateral dimensions). Gradually the entire size of the caudal segments becomes markedly reduced. The length and amount of mobility in the caudal region vary considerably among species, but all higher primates with external tails use them for balance on arboreal substrates.

D. Spinal Cord

The spinal cord is housed in the vertebral canal formed by the arches of adjacent vertebrae (Hines, 1933; Noback and Moskowitz, 1963). The meninges that surround the cord, as well as the relative positions of the roots of spinal nerves to their exits via intervertebral foramen, are similar to those of other mammals including humans.

E. Clinically Significant Features of Back and Spine Morphology

Clinically significant features of back and spine morphology include the relative positions of spines of the vertebrae and the relationship between the vertebral column and bony pelvis. Morphological features associated with obtaining samples of cerebrospinal fluid are discussed in the section on clinically significant features of the head and neck (Section VI,L). With this one exception, most of the bony landmarks used to locate internal structures in the cervical, thoracic, and abdominal regions are on the ventral surface and are discussed with the thorax, abdomen, and pelvis (Sections IX and X).

In the vertebral column the spines of the middle thoracic vertebrae, in particular, angle sharply caudally and the tip of a spine may actually be dorsal to the body of a vertebra caudal to it. The spines of the lumbar vertebrae are very broad in a cranial–caudal direction and are easily palpated until surrounded by the ilia. The ilia project cranially from the sacroiliac joints and the iliac crests usually lie approximately parallel to the inferior part of the second to the last lumbar vertebrae. Although most nonhuman primate anatomy is more similar to human anatomy than to other mammals, the bony pelvis and its relationship to the vertebral column is an exception. In this case the nonhuman primate bony anatomy is similar to that of other quadrupeds rather than to the human biped. The soft tissue, as discussed in the sections on the abdomen, pelvis, and perineum (Sections X and XI), however, is more similar to humans in many respects.

VIII. LIMB MORPHOLOGY

A. Overview of Limbs

The limbs of all primates follow the generalized mammalian pattern. Modification of this basic pattern in primates is usually not a change in the number of elements but rather a species difference in the relative curvature, robustness, and length of bones as well as migration of the positions of muscle attachments. Usually these differences in limb morphology are not based solely on evolutionary relationships but instead can be

correlated with functional similarities, or differences, in locomotor behavior and habitat use (Ashton and Oxnard, 1964).

The relative lengths of forelimbs, hindlimbs, and trunk are commonly used as basic indicators of the morphology of a particular species. All higher primates except the Callitrichidae have at least one pair of limbs longer than their trunk. As discussed in the section on back morphology (Section VII,A and C) there is considerable variability in the vertebral columns of primates, particularly in the lumbar region. Short vertebral columns are frequently found in the lesser and great apes and in species highly dependent on suspensory locomotion. Long vertebral columns are found most frequently in leaping species. In general, long limbs relative to trunk length are found among terrestrial quadrupedal primates, whereas relatively short limbs which lower the center of gravity are common among arboreal quadrupeds. Arboreal species whose locomotion emphasizes suspensory activities (brachiation, semibrachiation, or arm swinging) generally have relatively long forelimbs while species who engage in extensive vertical clinging and leaping usually have relatively long hindlimbs.

The relative lengths of forelimbs and hindlimbs (intermembral index = length humerus + radius × 100/length femur + tibia) also vary considerably and can be correlated to specific locomotor patterns (Fleagle, 1988; Schultz, 1969). Vertical clingers and leapers generally have low intermembral indices (50–80), indicating short forelimbs and long hindlimbs. Both terrestrial and arboreal quadrupeds generally have midrange intermembral indices (80–100) with forelimbs and hindlimbs approximately equal. Brachiators or species who use suspensory locomotion extensively usually have long forelimbs and short hindlimbs and high intermembral indices (100–150). These criteria are indicators of functional morphology but species variation does not always exactly follow these rules.

Externally all primate limbs appear to be similar but each species has its own unique modifications. All New World monkeys are basically arboreal and some of the species have prehensile tails that can be used as a fifth appendage (see the description of the tail in Section VII,A). Old World monkey limb morphology is generally divided into two subgroups based on whether the animals are primarily arboreal or terrestrial. Both lesser and great apes generally have long forelimbs relative to either their trunks or hindlimbs. All have morphology that is consistent with brachiators even though this mode of locomotion is not usually used by larger, heavier adults.

The hands and feet of all higher primates have marked prehensibility, i.e., all the digits can converge during flexion and diverge during extension (Figs. 3 and 4). Except in Callitrichidae, the thumbs (pollices) and big toes (halluces) of all higher nonhuman primates are functionally opposable, i.e., the palmar surface of the distal segment of the thumb or big toe can be placed parallel and opposite the equivalent part of one or more of the other digits. The hands of *Colobus, Ateles,* and *Brachyteles* are exceptions to this in that they lack functional thumbs. Old World monkeys and apes have true opposability of the thumb since the carpometacarpal joint allows longitudinal rotation of the first metacarpal. Cebidae New World monkeys have pseudo-opposability of the thumb because the carpometacarpal joints of these species do not allow longitudinal rotation of the first metacarpal. The big toes of all higher nonhuman primates are pseudo-opposable due to the lack of longitudinal rotation in their tarsometatarsal joints.

The length, both relative and numerically, of individual digits on the hands and feet show considerable variation among species. The pattern seen in each species appears to be well-adapted to its particular habitat use yet similar characteristics may be manifest in species with very different patterns of behavior. For example, in three species external thumbs are either severely reduced or absent even though the internal morphology is maintained. One of these, *Colobus,* is an Old World monkey which locomotes quadrupedally. The other two, *Ateles* and *Brachyteles,* are prehensile-tailed New World monkeys which make extensive use of suspensory locomotion. Other external modifications of the hands and feet of New World monkeys are found among the Callitrichidae. Unlike all other higher primates, Callitrichidae possess nails only on their big toes while the rest of their distal phalanges have claws (see the description of nails and claws in Section V,C).

In addition to locomotion the primate hand, and sometimes also the foot, is routinely used in other important aspects of the behavioral repertoire, e.g., feeding, social grooming, and sexual behavior. Its functional morphology thus reflects not only locomotor skills but also such things as dexterity in food acquisition. Although not studied extensively, some nonhuman primates display handedness, i.e., most rhesus macaques in a study population preferentially used one hand but the population was not predominantly left or right handed (Rawlins, 1995).

B. Musculature of Forelimb

The muscles of the forelimb of higher nonhuman primates are very similar to those of other mammals in general and humans in particular. [See Howell and Straus (1933a) for detailed descriptions of the muscles in rhesus macaques.] The orientation of muscles in the shoulder region differs slightly between quadrupedal primates and humans, but the overall similarities are much greater than the differences.

The distal segments of all primate forelimbs reflect a single pattern for the muscles of the hand. The extrinsic muscles of the hand are well-developed and the fibers for each individual digit are usually clearly defined. The intrinsic muscles of the hand are also well-developed with a typical digit receiving one muscle from each of the four intrinsic groups (e.g., lumbricals, contrahentes, palmar interossei, and dorsal interossei). The side and position of each insertion are dependent on the function performed by that particular muscle grouping. The development of the thenar and hypothenar eminences are closely correlated with the degree and strength of individual movements

routinely performed by the thumb and little finger respectively. The functional division of the hand is generally between the thumb (digit I) and index finger (digit II) but some higher primates routinely, or in particular modes of locomotion, use a functional division between digits II and III (e.g., *Alouatta*).

C. Musculature of Hindlimb

The muscles of the hindlimb are similar to those of other mammals and humans. [See Howell and Straus (1933a) or Stern (1971) for detailed descriptions of the muscles.] The names commonly used for identifying muscles in nonhuman primates, however, are the same as those used for humans. For example, the three glutei muscles are called mm. gluteus maximus, g. medius, and g. minimus in both human and nonhuman primates rather than mm. gluteus superficialis, g. medius, and g. profundus as in most mammals. Despite the use of the same terminology, the orientation of the muscles in nonhuman primates, particularly in the proximal part of the limb, differs from that of humans because of the latter's unique adaptation to bipedalism.

Like the hand, the foot of nonhuman primates has considerable dexterity. In the foot both the extrinsic and intrinsic muscles are well-developed and, as in the hand, the fibers for each individual digit are usually clearly distinguishable. The same four layers of intrinsic muscles seen on the palmar surface of the hand appear on the plantar surface of the foot. The size of the eminences, which are the equivalent of the thenar and hypothenar eminences in the hand, differs greatly among species and can be correlated to gait as well as habitat use. The big toe of all nonhuman primates is divergent from the other four digits and as such its musculature is generally well-developed. The length and robustness of the big toe vary greatly among species. In general, differences between the hand and foot are in the degree of development or independence of the digits with the exception that, unlike the hand, the foot has intrinsic extensor muscles on its dorsum.

D. Skeleton of Forelimb

Like the muscles, the bones of the extremities of nonhuman primates follow the mammalian pattern in general and humans in particular (Sullivan, 1933). The girdles, particularly the pectoral girdle, show some modification from the generalized mammalian pattern but are fairly consistent within primates. The proximal segment of each limb always has one bony element, the more distal segment has two discrete bony elements, the carpus has nine (or eight) and the tarsus seven short cancellous bones, the metacarpus and metatarsus each have five long bones per extremity, and each first digit has two phalanges whereas all of the other four digits have three phalanges.

The bones of the forelimb differ from those of many other mammals primarily in the presence of a well-developed, usually robust, clavicle. The clavicle articulates with both the manubrium of the sternum and the acromion process of the scapula and is a fully developed long bone in the adult. The scapula is well-developed and may be positioned more dorsally in primates than in other quadrupeds. These characteristics of the pectoral girdle correlate with the broadening and flattening of the thoracic cavity which is progressive within the primate order. The shape of the humerus, particularly the direction of the head and the longitudinal rotation within the shaft, reflects the locomotor behavior of the species. The ulna and radius are distinct bones whose articulations allow for considerable longitudinal rotation of the forearm. The amount of pronation and supination of the forearm varies greatly among species and can be correlated with locomotor behavior.

The carpus is composed of eight or nine short bones that are roughly arranged in two rows (Fig. 3). In most nonhuman primates the os centrale is a separate bone but in some it is fused with the scaphoid. The names of the other eight carpal bones are the same as those in humans: scaphoid, lunate, triquetrum, and pisiform in the proximal row from lateral (radial) to medial (ulnar) and trapezium, trapezoid, capitate, and hamate in the distal row from lateral to medial. As in humans the carpus is markedly concave on the palmar surface. This forms a deep carpal tunnel which transmits both the extrinsic muscles and the major vessels and nerves of the hand. The nonhuman primate carpus differs markedly from that of man in only three respects: (1) the regular presence of an os centrale, (2) the large size of the pisiform relative to the triquetrum, and (3) the presence of a large sesamoid bone near the trapezium.

The metacarpals and phalanges are all well-developed long bones and contribute to a highly mobile and prehensile hand. Other than robustness and relative lengths, the long bones of the hands of higher primates differ little from those of humans except for: (1) the carpometacarpal joint of the thumb in Cebidae, (2) the vestigial nature of the thumb in three species, and (3) the claws on the distal phalanges of Callitrichidae (see the descriptions of hands and digits in Section VIII,A and B). A further osseous difference between humans and nonhuman primates is that sesamoid bones are frequently found in the tendons of muscles in the hands of the latter.

E. Skeleton of Hindlimb

The bones of the hindlimb include a pelvic girdle that is very similar to that of other quadrupedal mammals (Sullivan, 1933) and differs markedly from the short, wide pelvic girdle of humans. The pelvic girdle is formed by the two os coxae which articulate on either side of the sacrum. Each os coxae is formed by the fusion of usually four bones: the ilium, ischium, and pubis as well as a fourth small bone, the acetabula. This latter bone fuses very early with the ilium and is frequently considered a part of it. The long, narrow blades of the ilia lie in parasagittal planes and give the pelvis a typically mammalian

quadrupedal orientation, i.e., the pubic symphysis is markedly caudal to the plane of the sacral promontory. In Old World monkeys and lesser apes the ischial tuberosities are covered by specialized skin called ischial callosities (for further discussion of these see Section V,B). The bony configuration of the pelvic girdle in nonhuman primates makes it one of the few areas of the body where their anatomy is more similar to other types of mammals than to humans. This similarity, however, is limited to the bony pelvis and the skeletal muscles which attach to it since the internal organs of nonhuman primates more closely resemble those of humans than of other mammals (see Sections X and XI).

The femur is the single bone in the proximal segment of the hindlimb. The shape and robustness of the bone vary among species depending on locomotor pattern but almost all higher primates lack a third trochanter. The tibia and fibula of the more distal segment are both well-developed, independent long bones which articulate both proximally and distally. The shape of these two bones as well as that of the associated patella are very similar to those in humans.

The tarsus of nonhuman primates is composed of seven short, cancellous bones: the talus, navicular, calcaneus, cuboid, and three cuneiforms, lateral, middle, and medial (Fig. 4). The names used for the tarsal bones of all primates, including humans, differ somewhat from those used for other orders. The distinct heel process (calcaneal tuberosity) on the proximal end of the calcaneus is a point of substrate contact in many patterns of locomotion and/or resting.

The metatarsals and phalanges of the foot are typical well-developed long bones. The mobility of all of the digits as well as the divergent, pseudo-opposable big toe and other modifications are described in the sections on feet and digits (Section VIII,A and C). In humans the convergence of the big toe as part of the development of a bipedal striding gait has resulted in numerous differences between the foot of humans and that of nonhuman primates. Despite this, the foot of nonhuman primates is still in many ways more similar to that of humans than to other highly specialized animals. As in the hand, the nonhuman primate foot and entire lower limb have more numerous sesamoid bones than the equivalent muscle tendons in humans.

F. Joints of Forelimb

The joints of the forelimb and hindlimb of nonhuman primates (Sullivan, 1933) resemble very closely the equivalent joints in humans. The primary difference between many of these joints and those of other mammals is a larger range of mobility. Unlike in many other animals, the limbs of primates are used in numerous other activities (e.g., feeding and social behavior) in addition to locomotion and thus they are not usually as highly specialized for a single repetitive motion (Oxnard, 1973). The actual range of motion permitted in any given joint is species specific and is influenced by age, sex, and environment (DeRousseau *et al.*, 1986; Turnquist, 1983; Turnquist and Kessler, 1989b).

The shoulder region consists of three synovial joints and one muscular complex which acts as a joint. The muscular complex which acts a joint is the movement of the scapula upon the thorax. This movement is without any direct bony articulations and includes movements in three planes (protraction and retraction, elevation and depression, and rotary movements which result in the glenoid fossa moving in the direction contrary to the movement of the inferior angle of the scapula). The sternoclavicular joint is a synovial joint with an articular disc within the capsule. Movement of the clavicle includes elevation and depression, retraction and protraction, and rotation around the long axis. Mobility in this joint is a critical component of most of the movements of the forelimb as a whole. A second synovial joint is the acromioclavicular joint which unites the scapula and clavicle at the lateral margin of the shoulder immediately superior to the glenohumeral joint. As in the previous articulation, unrestricted movement in this joint is essential for a large variety of movements of the extremity. The last synovial joint in this region is the glenohumeral or shoulder joint which is the articulation between the pectoral girdle and the proximal limb segment. Movement within the joint capsule is free but movement in this joint must be accompanied by movement in the other three areas in order for a full range of motion to be accomplished. The full range of motion in the shoulder region of nonhuman primates is not very different than that of humans. (See any functional anatomical text of human anatomy for a more detailed explanation of the movements and functioning of the shoulder region.)

The elbow joint capsule includes both the elbow joint and the proximal radioulnar articulation. The degree of extension and flexion in the elbow joint varies among species but in all cases the elbow joint strongly resembles that of humans both in configuration and in range of motion. Collateral ligaments attach broadly on either side of the joint and allow a larger range of extension than found in most other mammals.

The radioulnar joints include a proximal articulation enclosed in the same capsule as the elbow joint and a distal articulation whose capsule communicates with that of the wrist. In most primates both the radius and ulna articulate with the proximal carpal row, but in Hominoidea the head of the ulna is separated from the carpus by an articular disc and thus does not participate directly in the articulation between the forearm and hand (Lewis, 1972, 1974). Between the two radioulnar synovial articulations lies a strong interosseous membrane. The combined movement of the three areas results in pronation and supination of the forearm. The actual range of pronation and supination varies widely among species but can be correlated with locomotor patterns and habitat use.

The wrist joints include the articulation between the distal forearm and the proximal carpal row as well as the intercarpal articulations and articulations between the distal carpal row and the metacarpals. In Hominoidea the head of the ulna does not

directly participate in the wrist joint articulation since it lies proximal to an articular disc but the styloid process of the ulna may articulate with the pisiform (Lewis, 1972, 1974). The capsule surrounding this entire region may be subdivided into discrete parts but communications between them are common. The major ligaments for the joints are on the palmar side. The ligaments on the dorsum are relatively thin to allow for more mobility in the palmar direction. All of the joints in the region combine together to produce routine movements of the hand. The appearance of rotation of the hand is primarily supination and pronation of the forearm which may be supplemented by rotation in the midcarpal region in some species (Jenkins, 1981). Adduction (ulnar flexion or deviation) and abduction (radial flexion or deviation) occur primarily in the radiocarpal joint. Flexion (palmar or volar movement) and extension (dorsal movement) occur in both the radiocarpal and midcarpal joints. The two degrees of freedom and relative movement of bones of the wrist region of nonhuman primates are nearly identical to that of humans.

The carpometacarpal joints of nonhuman primates closely resemble those of humans. The joint capsules usually communicate with the capsules surrounding the carpus. The range of motion in the carpometacarpal joints of the four medial (ulnar) digits is similar to humans. Metacarpals II and III have very limited mobility whereas metacarpal IV and particularly metacarpal V are capable of flexion and limited extension. This morphology allows flexion of the ulnar side of the metacarpus and is consistent with the ability of primates to close their prehensile hands to firmly grasp relatively small objects.

The carpometacarpal joint of digit I (thumb) in Old World monkeys and great apes is very similar to that of humans. It is a saddle joint which allows movement in three planes and thus permits opposability of the thumb. Lesser apes also have an opposable thumb but the carpometacarpal joint more closely resembles a ball and socket joint. The Cebidae New World monkeys lack the ability to longitudinally rotate the thumb at the carpometacarpal joint and thus lack true opposability of the thumb. The palmar concavity of the hand and the separation of digit I from the other digits, however, permit pseudo-opposability of the thumb in these species. (See also the description of hands in Section VIII,A, B, and D.) Only the Callitrichidae of the higher primates lacks the ability to functionally oppose the thumb.

The metacarpophalangeal joints of the digits allow movement in two planes. These joints are all very similar to each other and are almost identical to those in humans. The characteristic prehensile hand of primates includes the ability to adduct (converge) the digits in flexion and abduct (diverge) the digits in extension. This ability is reflected in the configuration of the collateral ligaments of the metacarpophalangeal joints. The proximal extreme of the ligament is positioned more dorsally than that of the distal extreme. Thus the ligaments become taut in flexion, thereby limiting lateral movement, and lax in extension, thereby facilitating lateral movement.

The interphalangeal joints of the digits of the hand allow motion in only one plane and are nearly identical to each other and to humans.

G. Joints of Hindlimb

The sacroiliac joints are synovial joints between the alla of the sacrum and the ilium on either side. The joint normally has very little movement and is supported strongly by numerous ligaments. Anteriorly the two sides of the pelvis articulate at the pubic symphysis. As in other quadrupeds, this symphysis is long and includes a considerable part of the ischium as well as the inferior ramus of the pubis. In nonhuman primates the articulations of the pelvic girdle closely resemble those in other quadrupeds and are dissimilar to those in humans, particularly in their shape. Degenerative changes in the pubic symphysis of nonhuman primates are similar to humans and have been correlated with age, sex, and parity (Rawlins, 1975; Tague, 1990).

The hip joint of nonhuman primates is similar to that of other quadrupeds in its orientation to the pelvis. The soft tissue and range of motion in three planes, however, are not markedly different from those in humans with the exception of the increased extension necessary to maintain the bipedal striding gait of humans. As in humans, the spiral arrangement of the ligaments in the capsule permits the range of abduction–adduction and mediolateral rotation of the hip to be greater during flexion than during extension. The actual amount of movement in any plane varies among species and can be correlated to behavior. No nonhuman primate has the degree of hip extension and the ability to sustain upright posture which are seen in humans.

The knee (stifle) joint in nonhuman primates is nearly identical to that of humans in both structure and motion. The patella, two meniscii, and the collateral and cruciate ligaments lie in the same positions. As in humans, some rotation is permitted when the joint is flexed but not when the joint is extended. The knee joint of nonhuman primates usually does not fully extend to 180° but the range of motion in flexion and extension is always considerable.

The proximal and distal tibiofibular joints are the articulations between two distinct, well-developed bones. As in humans, neither of the two joints permit much movement. The predominant fiber direction of the interosseous membrane which connects the shafts of the two bones reflects the direction of the predominant forces during locomotion.

The ankle (talocrural) joint is primarily the articulation between the tibia and the talus but the distal fibula contributes to the lateral aspect of the articulation (the lateral malleolus). The morphology of this joint in nonhuman primates is similar to that of humans except that limited rotation is possible in most species. The joint capsule of the ankle joint is usually separate from that of the rest of the tarsal region.

The tarsal joints can be subdivided into two basic groups according to their positions. The first group is the talocalcaneal

joints which lie between these two bones. The second group is collectively called the transverse tarsal joint. This is the general name used for the joints between the talus and calcaneus and the more distal bones. The configuration of all of these joints, the continuity of the joint capsules, and the arrangement of the ligaments are very similar to those in humans. The major difference between the tarsal regions of humans and nonhuman primates is the presence of permanent longitudinal and transverse arches in humans that are integral parts of his adaptation to a striding gait. The foot of nonhuman primates lacks these semirigid arches and has a plantar concavity similar to that seen in the hand. As in the hand, foot movements involve a variety of joints. Plantarflexion and dorsiflexion occur primarily in the talocrural joint, abduction and adduction occur in the talocalcaneal and talocrural joints, rotation occurs in the talocalcaneal and transverse tarsal joints, and inversion and eversion occur in all of the joints.

The tarsometatarsal joints are very similar to the carpometacarpal joints for the lateral four digits (digits II, III, IV, and V). The big toe of nonhuman primates is divergent and the tarsometatarsal joint of digit I thus permits considerable mobility in two planes. The joint, however, does not freely permit longitudinal rotation of the metatarsal and thus the big toe is only pseudo-opposable.

The metatarsophalangeal joints are similar to the metacarpophalangeal joints and show a similar pattern for digit divergence and covergence. The motions permitted are plantar flexion, dorsiflexion, abduction (divergence), and adduction (convergence). Depending on the species, some nonhuman primates use their feet much like hands. This region of the nonhuman primate foot more closely resembles the equivalent area in the hand rather than the bipedally adapted foot of humans.

The interphalangeal joints of the toes are very similar to those of the hand and have very similar planes of motion.

H. Neurovascular Systems of Limbs

The nerves of the forelimb (Howell and Straus, 1933b; Swindler and Wood, 1973) are usually derived from the ventral rami of spinal nerves C5, C6, C7, C8, and T1. The basic pattern for the development of the brachial plexus and the final distribution of the peripheral nerves is the same in all primates both nonhuman and human. The position of the nerves as they course through the forelimb follows the same general pattern in all mammals. The names for nerves in nonhuman primates are the same as those in humans if these differ from those used in other animals.

The nerves of the hindlimb are usually derived from ventral rami of spinal nerves from all lumbar segments as well as the first two sacral segments. Despite the fact that the number of segments in these two regions varies considerably between nonhuman primates and humans, the basic pattern for the development of the lumbosacral plexus and the final distribution of the peripheral nerves does not differ. The number of actual nerves contributing to the plexus is usually the same in all the species. The position of the nerves as they course through the hindlimb follow the same general pattern in all mammals. As in the forelimb, the names of the nerves in nonhuman primates are the same as those in humans if these differ from those used in other animals.

The blood supply to the forelimbs and hindlimbs of nonhuman primates (Lineback, 1933b) is the same as in humans and other animals. The primary source of blood for the forelimb is the axillary artery and for the hindlimb the external iliac artery. The course of the vessels in the limbs resembles that of humans as well as other animals. Particularly because of the similarities in the hands and feet, the descriptions of the vascular supply of humans are applicable to nonhuman primates.

I. Clinically Significant Features of Limb Morphology

Clinically significant features of limb morphology in nonhuman primates include awareness of multiple uses of the extremities, locations for venopuncture and peripheral nerve biopsy, and rate of epiphyseal fusion of the long bones as a means of aging immature animals (see Section IV,B).

The extremities of nonhuman primates are morphologically and functionally more similar to human limbs than to other animals. For this reason books on human orthopedics and surgical anatomy are better guides than those standardly used in veterinary practice. One of the most important concepts regarding primate extremities is that they are not unipurpose. They have therefore retained a generalized form to enable the animal to manifest a wide range of behaviors. In addition to large ranges of motion in the anterior–posterior plane both extremities also have considerable mobility in other planes. The forelimb (upper) in particular can usually be markedly abducted and longitudinally rotated. The supination and pronation of the forearm and the dexterity of the hand add yet other dimensions to mobility. The full variety and range of motion for each extremity is important not just for locomotion but also for feeding, grooming, and other forms of social behavior. In primates the functional significance of the extremities is much greater than simply a means to translocate the body.

Peripheral nerve biopsies can be readily obtained from branches of the cutaneous sural (lateral) nerve. These sensory branches pierce the deep fascia of the lateral distal thigh and proximal leg. As in humans, a large branch usually parallels a tributary of the saphenous vein on the posterior aspect of the leg and thus is accessible for biopsy.

Venopuncture and arteriopuncture in nonhuman primates usually utilize the femoral vessels immediately inferior (distal) to the inguinal ligament. This ligament is palpable between the anterior superior iliac spine and the pubic tubercle. The pulse of the femoral artery is readily palpable and the vein lies immediately lateral to the artery. Another site frequently used for

venopuncture, and the most common site for the introduction of intravenous fluids, is one of the tributaries of the saphenous vein which courses up the midline of the posterior aspect of the leg. In the literature on humans this vein, which passes by the medial malleolus, is called the short (small) saphenous vein. It is equivalent to the caudal branch of the medial saphenous vein described in most veterinary literature.

IX. THORACIC MORPHOLOGY

A. External Morphology and Position of Organs

The external thoracic morphology of nonhuman primates differs from that of most quadrupeds primarily in the shape of the thorax. The primate thorax has been progressively flattened dorsoventrally with a concurrent repositioning of the scapula and ultimately an increase in the circumductual potential of the humerus. The nipples of the single pair of mammary glands in nonhuman primates generally approximate the level of the sixth ribs as they approach the sternum. The length of the nipples greatly increases following lactation and it is not uncommon for them to be markedly asymmetrical. The secretory cells of the mammary gland are widely distributed over most of the anterior thorax from the axilla to the inferior margin of the rib cage. Following lactation there is considerable involution of the glandular tissue in nonpregnant females, but in pregnant females there is little involution of the gland.

The dorsoventral flattening of the rib cage results in slight repositioning of the organs of the thorax. When relaxed, the respiratory diaphragm raises to a level slightly superior to the xiphoid process of the sternum. The heart lies in the middle mediastinum between the two pleural cavities. In this position the great vessels are oriented almost directly cranially rather than slightly dorsally as in quadrupeds with deeper rib cages.

B. Skeleton

Progressively more pronounced curvature of the ribs in nonhuman primates is correlated with the dorsoventral flattening of the thorax. In general the costal cartilages of the first eight ribs articulate directly with the sternum, the cartilages of the next two ribs usually attach to the costal cartilages of the superior rib, and the last two ribs are usually floating. (See the description of thoracic vertebrae in Section VII,C for the vertebral attachments of the ribs.) The sternum consists of the manubrium, the body which usually has five segments, and the xiphoid process. The clavicles articulate with the superolateral aspects of the manubrium to form the sternoclavicular joints. (See the descriptions of the joints of the shoulder region in Section VIII,F for details of this joint.) The first ribs articulate with the lateral margins of the manubrium, ribs 2 with the junction between the manubrium and the first segment of the body, ribs 3 between the next two segments, etc., and finally ribs 7 and 8 between the last segment of the body and the xiphoid process.

C. Musculature

Like the posterior thoracic wall, the intrinsic muscles of the anterior thoracic wall (Howell and Straus, 1933a) are overlain by muscles of the upper extremity which migrate into the area. In nonhuman primates these extrinsic muscles include the m. subclavius, m. panniculus carnosus, and three pectoral muscles: mm. pectoralis major, minor, and abdominalis. The m. subclavius is similar to that in humans because it passes between the first rib and the inferior surface of the clavicle. The m. panniculus carnosus arises from the superficial fascia of the entire lateral side of the torso and inserts into the humerus with the mm. pectoralis. The action of this muscle usually moves the skin of the side. There is tremendous variability in the extent and development of this muscle in primates but generally it decreases with ascent in the order culminating with its disappearance in humans. The three pectoral muscles in primates also show considerable variability. In general the m. pectoralis major is the equivalent of m. pectoralis superficialis in other mammals. This muscle arises broadly from the sternum and is similar to that in humans except that it lacks a clavicular head in most nonhuman primates. The mm. pectoralis minor and abdominis are equivalent to m. pectoralis profundus in other mammals. The primary difference between other mammals and nonhuman primates is that the deep pectoral layer is divided into two distinct muscles. In both Old and New World monkeys the m. pectoralis minor generally inserts onto the humerus or glenohumeral joint capsule. In apes (and humans) it generally inserts into the coracoid process of the scapula.

The intrinsic muscles of the thorax include the external and internal intercostal muscles as well as m. transversus thoracis. The extent of these muscles, the direction of their fibers, and the positions of the accompanying vessels and nerves in the superior margin of each intercostal space are similar to that of other mammals, including humans.

D. Diaphragm

The diaphragm is skeletal muscle which completely divides the thoracic and abdominal cavities. The crura of the diaphragm, the position of the central tendon, the hiatuses for the inferior vena cava, esophagus, and aorta, and its innervation and blood supply are all similar to other mammals, including humans.

E. Mediastinum and Autonomic Nerves

The mediastinum completely separates the right and left pleural cavities. As in humans and other mammals the esopha-

gus lies posterior to the roots of the lungs and the pericardium and anterior to the thoracic (descending) aorta. The dorsoventral narrowing of the thorax results in the mediastinum being less deep in nonhuman primates than in most quadrupeds and thus the esophagus lies in closer approximation to the posterior (dorsal) aspect of the pericardium. The course of nerves, arteries, veins, and lymphatic vessels (including the thoracic duct) in the mediastinum is similar to that of humans (Kuntz, 1933; Lineback, 1933b).

The autonomic nerves of the thorax include both sympathetic and parasympathetic fibers. The sympathetic trunks and ganglia lie ventrolateral to the vertebral bodies. Both white and gray rami communicantes pass between the trunks and the ventral rami. Preganglionic sympathetic fibers exit from the spinal cord and travel along white rami communicantes from spinal nerves T1 through L3 in most nonhuman primates. Preganglionic parasympathetic fibers to viscera in the thorax and abdomen travel in branches of the vagus nerves (cranial nerves X) whose central trunks pass posterior to the roots of the lungs and form the esophageal plexus before descending into the abdomen.

F. Cardiovascular System

The cardiovascular system of the thorax includes the heart and the eight great vessels as well as their numerous branches and tributaries. The relative development of the four chambers of the heart and the pattern of the coronary arteries and veins in nonhuman primates are very similar to humans. The major area of difference is that the number of leaflets in the atrioventricular valves may vary from two to four even within a single species whereas in humans the right side consistently has three leaflets and the left two. The three semilunar valves in the aorta and pulmonary arterial trunk appear to be constant in all species. The branches of the aortic arch and the thoracic (descending) aorta vary somewhat from species to species (DeGaris, 1935; Swindler and Wood, 1973) but are similar to other mammals, including humans. The innervation to the heart is similar to humans both in the location of nerves and in the origin of fibers (sympathetics from T1-5 and parasympathetics from cranial nerves X, the vagus).

G. Respiratory System

The respiratory system can be divided into upper and lower parts by the tracheolaryngeal junction (Lineback, 1933a). The nonhuman primate larynx opens from the distal pharynx and differs from that of humans in many respects. Despite this it is more similar to that of humans than to most other mammals (Kelemen, 1969; Negus, 1962). In a mature animal the hyoid is a complete single U-shaped bone with a marked concavity on the dorsal surface of the body. The size of the hyoid and the degree of concavity vary greatly between species and sexes. The largest development of the hyoid bone is found in the male New World howling monkey (*Alouatta* sp.).

As in humans the cricoid cartilage is the only complete tracheal ring in higher primates. Distal (inferior) to this, the posterior part of the tracheal cartilages is not fused and muscles and soft tissue form the posterior aspect of the trachea. The trachea bifurcates into the right and left bronchi high in the mediastinum. The right bronchus is more or less a direct continuation of the trachea whereas the left bronchus angles more markedly and is longer. The lungs of nonhuman primates differ from those of humans primarily in the number of lobes. The right lung of nonhuman primates frequently has a fourth (azygos or accessory) lobe which lies posterior to the inferior vena cava. The left lung generally has three lobes in rhesus macaques. A notable exception among nonhuman primates is the orangutan which reportedly has nonlobulated lungs (Lineback, 1933a).

H. Clinically Significant Features of Thoracic Morphology

Clinically significant features of the thorax include the position of the heart chambers, pulmonary recesses, esophagus, and trachea as well as the use of the ribs or sternum for bone marrow biopsies. The heart is displaced slightly to the left side of the thorax. The organ is rotated so that the right atrium and ventricle are more anterior and the left atrium and ventricle are more posterior. When viewed ventrally, however, the left ventricle is visible to the left of the right ventricle partly because of its hypertrophy. The arch of the aorta begins immediately to the left of the upper segments of the sternum.

The esophagus lies immediately posterior to the trachea in the superior mediastinum. In the inferior mediastinum it passes immediately posterior to the heart. The trachea lies on the midline and is separated from the sternum by the thymus gland which is large in young animals and involuted in older ones. The pleural recesses extend inferiorly (caudally) between the parietal pleura lining the lower ribs and the pleura reflecting over the superior (cranial) surface of the respiratory diaphragm. These recesses can be approached laterally through the inferior portion of the intercostal spaces if the probe is fairly shallow and does not penetrate the diaphragm. Both the ribs and sternum retain active hemopoietic tissue throughout the life cycle and thus bone marrow can be obtained for biopsy by entering the middle table of these flat bones.

X. ABDOMINAL AND PELVIC MORPHOLOGY

A. External Morphology and Position of Organs

The abdominal wall protects both the abdominal and pelvic viscera. The muscles of the abdominal wall (Howell and Straus,

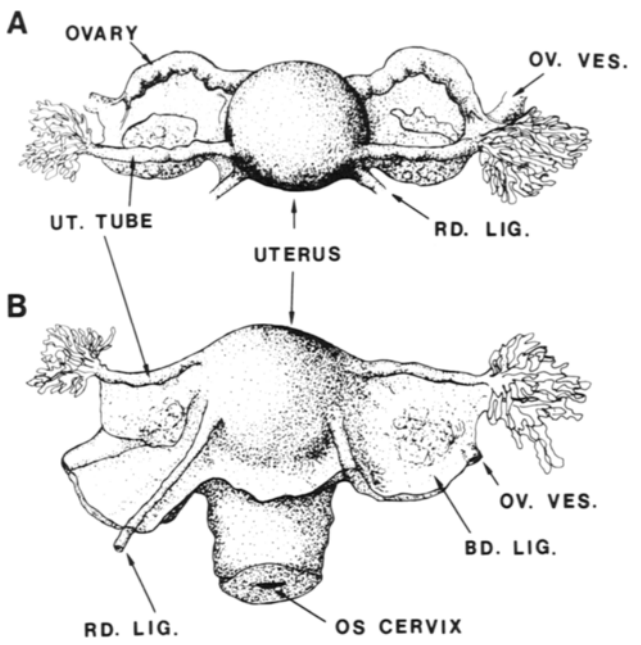

Fig. 11. Ovaries, uterine tubes, and uterus of a great ape (*Gorilla gorilla*). (A) Superior view; (B) anterior (ventral) view. Note the simplex uterus, the dorsal (posterior) position of the ovaries, the superolateral position of the ovarian vessels (ov.ves.), and the superolateral entrance of the uterine tubes (ut. tube) into the uterus. Peritoneal reflections cover the female genital system and form most of its ligaments, including the broad ligament (bd. lig.). The round ligament (rd. lig.) is a remnant of the gubernaculum and traverses the broad ligament between the uterus and the deep inguinal ring. The cervix projects into the anterior aspect of the superior vagina. Adapted from Wislocki (1932).

1933a) include the mm. rectus abdominis, obliquus abdominis externus and internus, and transversus abdominis. The fiber directions, positions, innervations, and vascular supply to these muscles are similar to other mammals, including humans. In primates the subcutaneous fat, which covers the muscular wall, is yellow as is most the other fat in the body.

The position of the organs in the abdomen and pelvis of nonhuman primates is very similar to that of humans. The stomach occupies the upper left quadrant and the liver occupies the upper right quadrant. The transverse colon is fused to the dorsal aspect of the greater omentum and lies immediately inferior (caudal) to the stomach. The spleen generally lies dorsally in the upper left quadrant of the abdomen adjacent to the fundus of the stomach. The kidneys are retroperitoneal and, unlike in humans, the left kidney lies considerably more caudal than the right one. The pancreas lies immediately dorsal (posterior) to the stomach and is oriented transversely. The pelvic reproductive organs of the female nonhuman primate differ markedly from those of most other mammals both in form and position. The uterus of both the human and nonhuman primate female is a simplex uterus (Fig. 11). The position of the female organs in monkeys and apes is very similar to that found in humans despite obvious differences in the pelves.

B. Peritoneal Cavity

The peritoneal cavity of nonhuman primates is divided into greater and lesser omental (peritoneal) sacs. As in other mammals these are the result of embryonic differential growth of mesenteries and the rotation of various parts of the digestive system. The greater omentum is suspended caudally from the greater curvature of the stomach and covers the transverse colon as well as the jejuneum, ileum, and most of the other abdominal organs. The lesser omentum lies between the stomach and proximal duodenum and the liver. Posterior (dorsal) to the lesser omentum and stomach and within the double folds of the greater omentum lies the lesser omental sac. The primary difference between humans and nonhuman primates in the locations of the contents and mesenteries of the peritoneal cavity is that the latter have more free mesenteries. In humans the mesentery of the duodenum from the middle of the first part on through the fourth part is fused. These segments are thus secondarily retroperitoneal as are both the ascending and descending colon. In nonhuman primates this secondary fusion is not as complete. In rhesus macaques only the first part of the duodenum and the first part of the colon lack free mesenteries.

C. Pelvis

The abdominal and pelvic cavities are bounded by the respiratory diaphragm (and inferior ribs) superiorly, the bony pelvis laterally, and the pelvic diaphragm inferiorly. The morphology of the vertebrae is described with the back (Section VII,C). The bony pelvis is described with the bones of the hindlimb (Section VIII,E). The shape of the bony pelvis in nonhuman primates is similar to that of other quadrupeds. The blades of the ilia are elongated in a cranial–caudal direction and the transverse plane of the pubic symphysis lies caudal to the plane of the sacroiliac joint.

D. Gastrointestinal System

The gastrointestinal system includes both the tubular gut and the associated digestive organs (Chivers and Hladik, 1980; Lineback, 1933a). The first part of this tube, the pharynx, is a shared pathway for both the respiratory and digestive systems. Caudally it continues directly into the esophagus. In nonhuman primates the pathway from mouth to pharynx to esophagus to stomach is almost a direct line. This differs considerably from humans where the pharynx is at right angles to the mouth.

All primates have cecums and relatively enlarged colons when compared to most mammals. All lack the ability to digest cellulose (Chivers and Hladik, 1980). Digestive system adaptations parallel dietary preferences and both can be correlated with overall body size (Fleagle, 1988). Smaller species tend to be insectivores whereas large species tend to be folivores. Frugivorous primates include the entire spectrum of body sizes.

Small frugivores frequently supplement their diet with insects whereas large frugivores supplement with leaves. Most of the adaptations of the digestive system seen in primates are found in folivores and occur in the stomach, cecum, and/or colon. In folivorous species the digestion of cellulose is performed by colonies of microorganisms living in dilated regions of the digestive system. Some folivorous Old World monkey groups such as Colobines have an enlarged stomach which allows for fermentation. The four part compartmentalization of their stomach however is not as complete as that seen in ruminants nor is there any regurgitation of food. Enlarged colons (and cecums) serve a similar function in folivorous New World monkeys, apes, and other Old World monkeys. A vermiform appendix is found only in apes (and humans).

The accessory digestive organs include the liver and pancreas. The spleen is not a digestive organ but it develops within the dorsal mesentery of the stomach and thus is closely associated with the gastrointestinal system. The liver is a large organ in the superior right quadrant of the abdomen which expands laterally to occupy an extensive region inferior (caudal) to the respiratory diaphragm. It has three large distinct lobes and two smaller ones. The connective tissue separation between the left lateral lobe and the central lobe is well defined but that separating the right lateral lobe is not. The quadrate and caudate lobes are small and the latter nearly surrounds the inferior vena cava. The relationships of the hepatoduodenal ligament, ligamentum teres, and other ligaments are similar to those of humans. The gallbladder lies inferior to the caudal surface of the central lobe. As in other mammals the pancreas develops as two parts, one each in the dorsal and ventral mesenteries. During development these two parts fuse to form a single pancreas. The pancreas lies posterior to the stomach and its long axis is positioned transversely. The head lies adjacent to the duodenum which receives the two pancreatic ducts. The tail lies near the spleen. The pancreas is fused to the posterior (dorsal) abdominal wall and, as in humans, is secondarily retroperitoneal. The spleen (lien) develops in the dorsal mesentery and is suspended by mesenteric connections to adjacent organs. It is situated in the posterior (dorsal) lateral aspect of the superior left quadrant of the abdomen and is structurally similar to that of humans.

E. Urinary System

The urinary system includes two bean-shaped kidneys, their ureters, the bladder, and the urethra (Lineback, 1933a). The urinary system of nonhuman primates resembles that of humans except that the left kidney is generally inferior (caudal) to the right kidney. This is probably due to the great development of the left lobe of the liver in nonhuman primates. The suprarenal (adrenal) glands (Miller and Leonard, 1933) are not part of the urinary system but they are situated on the superior poles of the kidneys as in humans. Details of the urethral openings are included in the description of the perineum (Section XI).

F. Female Genital System

The female genital system includes paired ovaries and uterine (fallopian) tubes whose medial ends enter into a midline simplex uterus (Fig. 11). In primates the female genital organs (Mossman, 1977; Wislocki, 1932) undergo considerable migration during development. In the nongravid adult they are positioned caudally in the pelvis where they lie inferior to the peritoneal sac. The major ligaments of the viscera are formed by reflections or folds of the peritoneum. The female genital system of nonhuman primates is very similar to the human female genital system, and very different morphologically from most other mammals. The ovaries are oval or fusiform and lie on the posterior layer of the broad ligament. Both ovulation and luteinization are spontaneous. The degree of convolution of the uterine tubes varies among species. The single midline uterus can be subdivided into a fundus, body, and cervix. The cervix protrudes into the anterior (ventral) superior portion of the vagina. The blood supply to the uterus includes extensive anastomoses between uterine and ovarian arteries.

As a general rule pregnancy in primate species results in a single infant, although twinning is common in some New World species. All primates have relatively long gestation periods and well-developed, precocious neonates. Birth occurs through ruptured membranes. The placenta and long umbilical cord remain functionally intact until the neonate begins to breathe. Fetuses which die before term are usually aborted, although occasional cases of "mummified" fetuses have been reported (Mossman, 1977). The fetal membranes of anthropoids differ from those of other mammals and include a hemochorial villous placenta, a rudimentary yolk sac, and a rudimentary allantoic vesicle if it is present at all (Luckett, 1974).

G. Male Genital System

The male genital system includes a pair of testes, ductus deferens, seminal vesicles, and bulbourethral (Cowper's) glands as well as two single midline structures, the prostate and urethra. The overall morphology of the spermatic cord and male genital system in nonhuman primates is similar to humans and most other mammals.

In the adult the testes are located in a scrotum outside the body cavity. In all primates the testes (Martin, 1990) descend into the scrotum before birth or shortly thereafter. Following this initial descent of the testes, there is considerable variation between primate species as to whether or not they stay in the scrotum prior to puberty. For example, in young rhesus macaques the immature testes actually ascend out of the scrotum and reenter the inguinal canal to redescend nearer puberty (Wislocki, 1933). Once puberty is passed the testes do not reenter the body cavity and the inguinal canal collapses. In species with well-defined nonbreeding seasons the testes of nonsexually active adult males may ascend to the external inguinal ring and the scrotum

may contract. Conversely during the breeding season testes volume may increase (Conaway and Sade, 1965; Sade, 1964).

Ductus deferens enter the body from each testes via the inguinal canals and course medially inferior to the bladder. Paired seminal vesicles communicate with the ductus deferens near where they enter the prostate gland. The prostate gland in nonhuman primates is more conspicuous than in some other mammals but it does not form a complete ring around the urethra, i.e., it lacks the anterior lobe found in humans. The final pair of glands, the bulbourethral glands, enter the urethra just caudal (inferior) to the urogenital diaphragm (m. sphincter urethrae). [See the section on perineal morphology (Section XI) for further details of external genitalia.]

H. Neurovascular Systems

The autonomic nervous system in the abdomen and pelvis includes sympathetic nerve fibers which exit the spinal cord with spinal nerves T6 through usually L3 (Kuntz, 1933). The parasympathetic nerves to the gut proximal to and including the first two parts of the large intestine are from the 10th cranial nerves (vagus). The parasympathetic nerves to the more distal gut and to pelvic viscera generally arise with spinal nerves L7 through S2. As in humans and other animals it is not uncommon to find the levels of nerves exiting for the autonomic nervous system to vary by one level in either direction.

The cardiovascular system in the abdomen and pelvis follows the general pattern seen in all mammals (Lineback, 1933b). The exact location of the vessels and relative positions of the portal and vena caval systems are similar to those of humans as well as most mammalian quadrupeds.

I. Clinically Significant Features of Abdominal and Pelvic Morphology

Clinically significant features of abdominal and pelvic morphology include the location of the pancreas for pancreatic biopsy, structures encountered in gastric intubation, and palpation of pregnancy.

The most common site for pancreatic biopsies is from the tail of the organ. The entire organ lies retroperitoneal immediately posterior to the stomach. Its long axis lies transversely and its head is surrounded by the duodenum. The pancreas is triangular in cross section with the broadest base facing anteriorly (ventrally). The tail of the pancreas lies dorsally in the upper left quadrant of the abdomen and is accessible when approached laterally. Pancreatic biopsies can be obtained through a vertical left paramedian, paracostal incision. The tail of the pancreas can then be dissected out from its position near the spleen.

Gastric intubation of nonhuman primates is similar to other quadrupeds and is usually performed nasogastrically to prevent biting on the tube. Elevation of the chin straightens the pharynx and results in a fairly straight line between the pharynx, esophagus, and stomach. The distal (lower) part of the esophagus is surrounded by a thick layer of circular muscle which acts as a valve and may offer resistance to dilation. The stomach itself is relatively large and offers no resistance to intubation.

Palpation for pregnancy, even early pregnancy, is possible because the uterus is a single midline structure lying superior (cranial) to the bladder. Through the abdominal wall even the nongravid uterus is palpable between the thumb and four fingers immediately superior and posterior to the pubic symphysis. Alternatively, rectal digital palpation can be used. Determination of the age of the pregnancy is based on uterine texture and fetal size which varies considerably among species.

XI. PERINEAL MORPHOLOGY

A. Overview of Perineum

The perineum of nonhuman primates differs from humans and shows considerable variation among species. In addition, some species have marked seasonal variation in coloration, prominence of sexual organs, and/or swelling of the skin of the perineum (Wislock, 1933). The perineum is bounded by the base of the tail dorsally and the pubic symphysis ventrally. The lateral extremes of the perineum are the ischial tuberosities which in some primates are covered by ischial calosities. (These are described in detail at the end of Section V,B.) The perineum includes all of the soft tissue caudal to the pelvic diaphragm.

The deep perineum of both sexes includes the muscular external anal sphincter and the urogenital diaphragm which includes the sphincter for the urethra. The roots of all the external genitalia are also found in this area and thus it is traversed by the continuations of various parts of the genital systems of each sex. The structures of the external genitalia are similar to those of humans and other mammals. Well-defined columns of erectile tissue are evident in both the penis and clitoris. The amount of development, particularly of the roots of the external genitalia, is species specific. The nerves and arteries of the area are similar to those of humans and most other mammals.

B. Female Perineum

Externally the female perineum incorporates the external manifestation of the clitoris, the orifice of the urethra, the vaginal opening, and the anus. Nonhuman primates do not have long urogenital sinuses. The urethra enters the vestibule near the vagina. At various times the vulva may be plugged either by copulatory seminal plugs or during pregnancy. The size of the labial folds and clitoris varies tremendously among species. The most extreme example is in *Ateles* where the clitoris of the adult female is more prominent and pendulous than the penis of the

adult male. In this species the clitoris is often mistaken for a penis by novice observers.

In many species of nonhuman primates the skin of the female perineum may become swollen, puckered, and highly colored around the time of ovulation and/or to a lesser extent in late pregnancy. The swollen area is termed the sexual skin. The extent of swelling and color change varies considerably among species. It may involve extensive areas from the pubic symphysis to the base of the tail as well as the adjacent posterior thigh. The amount of fluid accumulated in sexual skin in the perineum is enormous in some species.

C. Male Perineum

The male perineum externally includes the anus, scrotum, and penis. In some species the perineum may be brightly colored, particularly during the breeding season. For example, the anal triangle is bright red and the scrotum bright blue in the adult *Erythrocebus patas*. The penis of all nonhuman primates is pendulous but part of the shaft may be fixed to the ventral body wall by skin. Considerable species to species variation in the fixation of the penis and in the size and the side of deviation of the os baculum exists. The testes of all adult nonhuman primates are located in a well-developed scrotum. Descent of the testes into the scrotum is described in the section on the male genital system (Section X, G). The scrotum is pendulous, but it may contract during the nonbreeding season when the testes ascend toward the external (superficial) inguinal rings. The relatively anterior position of the scrotum and the fixation of the penis may make the penis appear relatively shorter in some species than in others. Although usually the scrotum is posterior (dorsal) to the penis, in a few species it may appear to be anterior (ventral) to the penis.

D. Clinically Significant Features of Perineal Morphology

Clinically significant features of perineal morphology include the effects of puberty and seasonal variation on the appearance of the perineum and prolapses of various organs.

In both female and male nonhuman primates, puberty (Pereira and Altmann, 1985) can affect the morphology of the perineum. Prior to puberty the female perineum remains constant in its appearance. In numerous species at the time of puberty and during every subsequent ovulatory cycle the perineum undergoes very distinctive, cyclic changes.

In the female perineum the morphological changes at the time of ovulation may include swelling of the sexual skin and the possible presence of a postcopulatory vaginal plug. The amount of fluid accumulation in sexual skin in some species, e.g., *Pan* and *Papio*, is so extensive that the skin may actually rupture. Without outside intervention these ruptures heal within a few days as turbescence subsides. Several weeks after ovulation menstrual bleeding may be evident from the vaginal opening. The length of the actual cycle varies among species. In species with distinct breeding seasons, the cyclic changes in the perineum are evident throughout the breeding season unless the female becomes pregnant. During the nonbreeding season there are fewer recognizable changes in the perineum except those correlated with late pregnancy.

In the male perineum, morphological changes occur prior to, or at the time of, puberty. The rate of these changes is species specific depending on when the testes finally definitively descend into the scrotum. In species (e.g., *Erythrocebus patas*) where the testes redescend late, the scrotum does not usually become pendulous until puberty. Prior to the redescent of the testes the scrotum is represented by folds of loose skin adjacent to the midline. Thus in these species the male perineum prior to puberty resembles that of the female. Similarly the penis, although pendulous at birth, may also retract prior to puberty. Once puberty is passed both the scrotum and penis remain pendulous.

Seasonal variations in the male perineum of species with distinct breeding seasons include ascent of the testes to positions closer to the external inguinal rings, partial contraction of the scrotum, and diminished coloration of the scrotum.

Prolapse of the bladder, vagina, uterus, and rectum are all seen in nonhuman primates. Prolapses are much more common in females than in males. The vagina, the uterus, and/or the bladder can all prolapse through the vaginal opening. These are all usually the result of weakness of the anterior vaginal wall. Prolapse of the rectum occurs in both sexes due to weakening of the muscles of the pelvic diaphragm or straining to defecate. Extreme swelling of the sexual skin in females can appear to be similar to the prolapse of visceral organs.

XII. CONCLUSIONS

The morphology of all primates follows a pattern which is generally that of an unspecialized mammal. The considerable variability of this pattern within the order can be grouped according to various evolutionary and/or functional criteria. The evolutionary divergence between the two suborders is reflected in morphological differences between prosimians and the more numerous and diverse anthropoids or higher primates.

The first major morphological grouping of higher primates is by geographical location, i.e., Old or New World. New World monkeys have three premolar teeth in each quadrant whereas all Old World higher primates have only two. All New World primates are monkeys and all are arboreal. Old World higher primates include humans and the great and lesser apes as well as monkeys. Some New World monkeys have prehensile tails whereas no Old World primates have true prehensile tails.

The second major morphological grouping of primates occurs within the Old World primates. Nonhuman Old World higher primates are subdivided into apes and monkeys. This

division is based on a number of criteria but the most obvious are the differences between primarily quadrupedal locomotors (Old World monkeys) and more mixed forms of locomotion (apes). The latter group is characterized by lengthening of the forelimbs and flattening of the thorax among other criteria.

These first two morphological groupings follow evolutionary lines. The next morphological grouping, however, cuts across evolutionary boundaries and is based on habitat use. The criteria here is whether the species is primarily arboreal or terrestrial. Arboreal monkeys tend to have relatively smaller body sizes, shorter limbs, and longer digits than their terrestrial counterparts. In morphology related directly to habitat use, arboreal monkeys in the Old and New World may more closely resemble each other than either resemble highly terrestrial Old World monkeys.

A last criteria which categorizes morphology of nonhuman primates is sexual differences. In some species there is little overall morphological difference between the sexes whereas in others there is a tremendous difference both in size and shape. Sexual differences tend to be greater in terrestrial than in arboreal monkeys. In species where there is sexual dimorphism it is usually evident in body size, hair coloration, and tooth size, particularly the length of the upper canine.

Nonhuman primates are, for the most part, quadrupeds, but morphologically they are more similar to humans, a member of the same primate order, than to most other quadrupeds. Even in the hindlimb where humans have evolved elaborate adaptations of the pelvis and foot to accommodate the bipedal striding gait, the differences are primarily in bony morphology and not in the soft tissue organs. For these reasons both surgical and orthopedic books written for medical instead of veterinary use are generally more informative for any evaluation of nonhuman primates. Clearly a certain degree of flexibility must be included before the material for humans can be applied to nonhuman primates such as rhesus monkeys. The basic information for humans, however, usually provides a better guide than the standard references for domestic animals.

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CHAPTER 5

Study of Primate Social Behavior

Lawrence E. Williams and Irwin S. Bernstein

I. INTRODUCTION

The task of defining all the social structures and organizations seen in the order Primates is a challenging assignment. The composition of social units is similarly variable from near solitary species to societies with multilayered organizations. Behavioral flexibility has permitted the various species to expand and occupy a multiplicity of ecological niches. Breeding systems range from monogamous, to possibly polyandrous sys-

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tems, to one-male units which may or may not be permeable to other males in the breeding season, through multi-male/multi-male groups with either one male subunits or polygamous mating, and finally to fission–fusion, community style groups. Within each of these categories the specifics of what constitutes the social organization for a given species may vary greatly.

It is beyond the scope of this chapter to present specific data on any given species or group of species, instead the reader will be presented with general behavioral concepts as they relate to primates. Research methodology and experimental design concepts related to behavioral analysis and the potential contribution that behavioral science can make to captive primate housing and breeding are discussed. The first section provides background into the nature of social behavior of primates and how it can be described. The section on research design reviews experimental methodology from a behavioral perspective, including a discussion of two common pitfalls in behavioral research, the use of antidotes and the use of initial observations to confirm a hypothesis.

The last two sections are general statements about the study and use of behavior techniques with a special reference to primates in a laboratory or experimental setting. The suggested applications are reflections of an author's own perspectives; a rapidly growing literature is available outlining the suggestions and ideas of the many people now interested in this area. This chapter is intended only as an introduction to rather than a synopsis of the available literature. Each species of primate has a unique set of social and environmental needs that must be met for the animal to thrive in the laboratory.

A knowledge of behavior is critical for anyone who plans to work with primates. Knowing how a species lives in its "natural environment" will help to establish appropriate captive environments. The major adaptive thrust in primate evolution has been the ability of these animals to interact socially and to use behavior to react to new environments. Hans Kummer (1971) has suggested that primates have few ecological specializations other than sociality. This means that primates may be expected to act jointly in response to environmental challenges. Providing these opportunities for joint social acts will help most primates adjust to a captive environment. Many primates will show physiological and psychological manifestations of stress in the absence of multiple social partners with which to interact (Barnett and Hemsworth, 1990; Levine and Coe, 1988; Pond and Rush, 1983). On the other hand, species of Callitrichidae, adapted to life in monogamous pairs, may not flourish when housed in large groups with multiple members of each sex present. In primates like langurs (*Presbytis*), callitrichids, and *Aotus*, a great deal of the infant care is provided by individuals other than the mother. An isolated female and infant may not thrive even though this reduces the chances of aggression toward the infant from other animals.

Behavioral analysis is not only important in the housing and experimental use of nonhuman primates, but can be equally important in a clinical or diagnostic setting. Animals that act atypical in a natural setting are the ones most likely to be targeted by predators. This leads to a stoicism among wild animals that makes spotting a sick animal very difficult. Familiarity with the behavior patterns of an animal and how it ordinarily reacts to the presence of certain stimuli may make it possible to recognize an ill animal before gross signs appear.

II. LEVELS OF BEHAVIORAL ANALYSIS

Tinbergen (1951, 1963) stated that biological processes, including behavior, should first be described in terms of observable structure and then analyzed to provide answers to questions of immediate causes, adaptive significance, ontogeny or development, and evolution. Each of these viewpoints focuses on the same act, but examines it from different perspectives. A behavioral ethogram is often used in the study of behavior and begins as a dictionary or catalog describing typical behavioral acts. Ethograms are often organized by listing together those acts that may serve similar functions, such as maternal care, reproduction, self-maintenance, or aggression. Building an ethogram allows for standardization in the discussion of the behavior of a species.

The first task in building an ethogram is to describe what the animal does. The physical movements individuals make and how they are related to each other to form sequences of actions is the basis for all later descriptions of social roles and organizations. Once single acts are described, investigations may then focus on sequences of actions or complex social interactions and patterns such as social roles and the structure of social groups. In examining the basic behavioral units and searching for the functional significance of behavior, it may appear that two or more actions occur in the same types of exchanges and appear to serve similar functions. Certain species of primates may stare at each other with their mouths gaping open. Certain others may flash their genitals at each other. These patterns, though very different visually, signal the same kind of information, hostility, to the appropriate receiver. When describing behavior it is possible to focus on the physical movements or postures the animal performs, for example, terms like open-mouth state or hip touch denote the actual movement the animal makes. An alternative is to describe these patterns in terms of their presumed functions or consequences. A stare that often proceeds a chase may be labeled a threat face or a grimace may be called a fear signal or submissive gesture. While one may begin by describing the structure of the behavior of the animal, it is often their function that is most interesting.

As explained by Tinbergen (1963), one can seek to find the immediate or proximal cause of behavior in the environmental events that serve as triggers or stimuli eliciting a behavioral act. This can be a social stimulus, such as the presence of a higher ranking animal or endogenous stimuli, such as neurochemical or hormonal fluctuations. In either case, the response is linked to an immediately proceeding event that leads to the subject

acting in a predictable way. Animals flee because they are chased.

A second level of "Why" is the function of the response; the adaptive significance the response holds for the animal. Adaptive significance is concerned with the ability of the animal to survive and produce offspring. Animals may yield to higher ranking group members because it is in their advantage to "short-cut" an aggressive encounter and not risk being seriously injured. When asking questions about adaptive significance, one is concerned with the biological function of an act. This function should in some way increase the fitness of an animal, i.e., its ability to survive and reproduce. This differs from the immediate cause and usually leads to confusion if two individuals are discussing an act at two different levels; one in terms of the immediately proceeding cause and the other in terms of future consequences to fitness. Aggression within a group of primates can be seen as the result of introducing unfamiliar animals to the same space or as the establishment of a dominance hierarchy which will better control the aggression and resource distribution within the group. Why a particular animal dominates others in access to food may be influenced by both the physical characteristics and social alliances of the individual. Why there are dominant animals is an entirely different question that must consider how the presence or absence of a dominance hierarchy has adapted the species to its environment (see Bernstein, 1981, for a discussion of dominance concepts).

The ontogeny of behavior is concerned with how responses or interactions with other animals change or develop over time. Beyond the physical development that allows animals to respond using more complex signals, the ontogeny of social behavior may also deal with how the animal is integrated into the society. One of the adaptations primates exhibit is an extended developmental period compared to other mammals of the same size. The dominance status of an individual may also be examined in terms of maturation or the history of an animal's experience, such as the support of a dominant or subordinate matriline. Socialization refers to the lifelong process by which primates learn from and adapt their behavior to the constraints of their social environment.

Since nonhuman primate behavior leaves few fossils, evolutionary questions are often approached by comparing similar responses in existing species. This comparative method uses morphological and behavioral data to determine which species are more derived and which are more conservative from within a group. It is then possible to infer evolutionary changes in behavioral patterns and to hypothesize selective pressures which have operated on a species.

III. DEFINITION OF PRIMATE SOCIETIES

A. Social Structure vs Social Organization

When studying the social behavior of primates it is important not to confuse social structure and social organization. On the one hand, social structure concerns the demography of a group of animals, the number of males per females, the reproductive patterns, territoriality, dietary limitation, and other information. Social organization, on the other hand, deals with the interrelations between individuals within a group. It should be obvious that social structure has a profound influence on the types of organization a group can exhibit. Species such as the organutan that are solitary and have long interbirth intervals may not rely on juvenile play groups to integrate younger animals into the group.

Feeding patterns can also influence the amounts and types of social interactions experienced by a species. Spider monkeys (*Ateles*) and howler monkeys (*Alouattinae*) have very different troop cohesion patterns that may be related to the type of fruit they eat. Spider monkeys feed on a fruit that is distributed in small, patchy clumps and therefore must spread out into small groups to forage, coming together to sleep (a fission–fusion type of structure). Howler monkeys move about in intact social groups, eating fruit that is abundant in large clusters.

Social organizations can be described in terms of the predictable patterns of interactions that occur within a group of animals. When patterns of social interaction serve specific functions they are often described as social roles (Bernstein and Sharp, 1965; Bramblett, 1973; Fairbanks *et al.*, 1978). Many individuals may serve similar roles (i.e., parental roles or group sentinel roles) and the same individual may serve multiple roles. The specific expression of each role varies from species to species reflecting the particular needs of individuals and the social structure of the group. Paternal behavioral is expressed quite differently by monogamous marmoset males and rhesus males living in a group consisting of many adult males and infants. In marmosets, males routinely carry infants over long distances, provide care for, and support sleeping infants. Although rhesus males occasionally may show some of these patterns, the paternal role consists largely of aggressive protection of distressed infants. Role differences can be described in terms of frequency and directionality of particular behavior patterns and social organizations can be thought of as the patterns of interactions among roles.

B. Evolution of Sociality

Sociality may have been selected genetically based on several advantages (Kleiman, 1977). The first is a lower susceptibility to predation. There is a statistical advantage to belonging to a group if a predator is attacking. The odds that a particular individual will be killed decrease with larger group size. Living as a "clumped resource" also makes it harder for the predator to detect and isolate its prey. A group of animals may be better able to detect and defend against predators.

Improved acquisition of food is a second advantage. Foraging activities that involve several cooperating individuals are more successful than solitary hunting. This is particularly true when food appears in rich clumps such as fruit on a tree or

insect swarms. Since primates are alert to the comings and goings of group members, social living might constitute a definite advantage. Food calls are a common occurrence in laboratory situations where food is usually clumped and abundant.

Social living provides for the sharing of limited resources. This extends beyond resources like food and water. Safe and suitable sleeping sites for hymadryas baboons are very limited. The ability to tolerate other conspecific individuals at close range facilitates the sharing of resources and aids in individual survival (Kummer, 1968).

These advantages are limited by other features in the environment. Resource distribution may act to limit the size of animal groups. Large groups of animals are easier for predators to track. Disease transmission is also a factor in limiting the size of social groups. These costs interact with the benefits listed earlier to place restraints on the size and composition of social groups. Taken together with phylogenetic constraints, these factors shape the social structures seen in different species.

C. Criteria for a Social Group

While there is no obvious tendency in social organization as one moves from prosimians to the apes, various authors have suggested that primate groups should meet the following five criteria to be considered a society (Eisenberg, 1965). First, they must show some form of temporal stability. Second, they should show a spatial unity throughout the time they are together. The scales used to measure these first two criteria need to be species relative. Chimpanzees typically express what has been termed a fission–fusion style of social structure (Goodall, 1973; Hiraiwa-Hasegawa *et al.*, 1984; Nishida, 1989). Subgroups of animals that frequently change members move about in a large, clearly defined area. Under these criteria this can only be considered a stable social organization if the time and space scales are much larger than those used for other primate species. For some galagos and lorises (Lorisidae) the spatial pattern is still harder to define. Although these species are rarely seen together, evidence shows that they should be considered as social animals because they preferentially associate with particular individuals when they are social.

This leads to a third criteria: members of a social group need to show a differentiation between group members and nonmembers, a type of group recognition. In the laboratory this typically manifests itself when animals are introduced into a stable social unit or even into a room of individually housed animals (Bernstein *et al.*, 1983; Southwick *et al.*, 1976; Williams and Abee, 1988).

The final criteria considers the interactions among the animals. A social group should have some form of communication system resulting in a coordination of activity. There should also be some form of division of labor, cooperation, or joint action on the environment. This cooperation might be joint defense against predators, joint exploitations of resource, or a division of labor as seen in the complex differential response patterns shown by individuals fulfilling role functions.

Most definitions of a society place an emphasis on the communication network established with the group. Kummer (1971) defined social groups as several individuals in close spatial proximity that interact more with each other than with outsiders. Altmann (1965) contended that once the channels of communication are established the other four characteristics of a society will follow. The animals must show temporal and spatial unity to communicate, and cooperation is a result of communication. Aggregations of animals are usually drawn together by external factors such as a concentration of food or spatial constrains such as laboratory caging. A social unit, on the other hand, consists of individuals who prefer to associate with one another. Understanding the differences between aggregations and social groups is important when managing captive groups of primates. Using the definitions outlined earlier, one can predict how groups of animals will react to changes in their environment. The establishment of social groups in the laboratory must recognize the social tendencies of a species. Once the group is established there will be a differentiation between group and nongroup animals expressed as an increase in cooperative, directed aggression toward newer animals. This is discussed later when we warn against simple addition of individuals to an established group (Section VII,A).

When studies involve captive animals, living in groups, much of this definition loses meaning. The animals necessarily communicate more among themselves than with other groups because they are restricted by the confines of their cages. However, it is possible to use the patterns of communication as the defining feature to study the relationships within a group (Williams, 1983). Using intragroup interactions, it is possible to study subgrouping and the various levels of primate societies. These studies emphasize the communication system within a group and the measures of intraspecific social responses, such as grooming, facial expression, and sexual responses. These responses are usually distinct and are easily quantifiable in terms of frequencies or durations when dealing with a small captive group. Even working with captive primates in large compounds may take a great deal of time in determining the exact social communication patterns using those responses mentioned earlier.

Analysis of the spatial arrangements of captive groups should give a preliminary indication of how a group of animals communicates and along what lines this communication occurs. This type of cliqual analysis assumes that if the animals are subgroups in a consistent pattern, some form of communication was required to establish these partner preferences. A nonsocial aggregation of animals should associate with each other in a random or nonsystematic fashion since no appreciable levels of organized communication, either agonistic or affiliative, are occurring. Social groups, on the other hand, would show more organized social responses. Since these responses are affiliative or agonistic, the animals should develop partner preferences

and their spatial distribution should reflect these preferences. Any radical change in normal social interaction patterns may presage an outbreak of aggression and spontaneous social reorganization which will demand management time.

D. Ecological Pressures on Primate Social Structures

Several theorists have attempted to organize primate social structures into conceptual models relating to various ecological contingencies. Crook and Gartlan (1966) attempted to classify diverse primate species based on correlations with variables they believed existed in the environment. They arranged the species they studied into four levels of social organization: nocturnal, diurnal–frugivorous–forest, diurnal–omnivorous–tree savannah, and diurnal–omnivorous–arid savannah. Another approach (Denham, 1971) took a different theoretical model. The influence of food density and distribution and of predation was dichotomized and all possible combinations of these variables were examined to predict sets of conditions. Space and resource allocation, sex ratios, and mating strategies were among the dependent variables. This model has an advantage over that of Crook and Gartlan in that it specified some variables associated with food as a resource and led to speculation on how these might relate to energy acquisition by the group.

Eisenberg *et al.* (1972) attempted to relate phylogenetic and ecological variables to one aspect of social structure, the degree of male involvement in group interactions. They postulated that adult males serve four roles in social groups: (1) maintain spacing between closed groups; (2) reduce competition by forcing out younger males; (3) protect the group; and (4) provide leadership by initiating and maintaining movement of the group. These functions were left to the males because the females were preoccupied with the protection and socialization of infants. Despite its wrong assumptions about females, this model does describe how a multi-male system might have evolved through the generalization of tolerance for sons in the group to other males that might appear. The adaptive significance of a multi-male group is evident in the increased protection against predators.

IV. GENERAL STATEMENT ON PRIMATE SOCIAL STRUCTURES

While general statements are difficult to make about primate social structures, most will fit into one of five broad categories: solitary, monogamous (possibly polyandrous systems), single-male/multi-female, multi-male/multi-female, and fission–fusion communities.

A. Solitary Primates

Species that forage largely solitarily with direct contact between the sexes occurring on an infrequent basis constitute the solitary primates. These include the Lorisidae and Tarsiidae, and the orangutan (Bearder and Martin, 1979; Charles-Dominique, 1977; Harcourt and Nash, 1986; Mitani, 1985a; Niemitz, 1984). The Lorisidae and Tarsiidae are nocturnal primates that range from Africa through southeast Asia. These animals have mating systems that range from monogamous to polygynous depending on the degree of home range overlap between the males and females. For many galago species the male has large home ranges that may overlap several females leading to polygynous mating. Male tarsiers, on the other hand, have ranges that tend to overlap only one female, a *de facto* monogamous pair, although there may be very little direct association between the sexes. Within these groups a large amount of variation exists in the amount of time spent in social contact. Organutans show a similar pattern of solitary foraging and infrequent associations. Adult males and subadult animals are most frequently seen as solitary animals, whereas adult females usually associate with several offspring.

B. Monogamy

Social groupings based in paired adults are widespread among the primates. Several lemur species, indirii and sifaka species, Callitrichidae (marmosets and tamarins), *Aotus*, *Callicebus*, and the lesser apes (gibbons and siamongs) all show some degree of monogamous social structure. Evidence (Terborgh and Goldizen, 1985), however, suggests that multiple males may live in the same family group in certain marmoset species, suggesting a flexibility in Callitrichidae groups not previously seen. Twinning in this genus places an extra burden on both parents. The costs of parental care are divided between members of the whole family unit. The causes of monogamy have been widely debated and are beyond the scope of this chapter. Because such a wide range of primates, prosimians, New and Old World monkeys, and apes display this as a pattern it may be assumed to have independently evolved several times within the order.

C. Single-Male/Multi-Female Groups

Groups in which a single male associates and with multiple female are necessarily more complex social organizations One male units are seen among the *Cercopithecus* (guenons) and *Colobinae* (Asian langurs and African colobus). Langur males will actively defend their units against all-male groups in transit. The aggression that typically is associated with the takeover of one of these groups has been correlated with incidences of infanticide (see Struhsaker and Leland, 1985, for a review). Within *Cercopithecus* there are at least two exceptions to the one-male rule. *Cercopithecus aethiops* have been recorded ranging from harem to large multi-male groups whereas *Cercopithecus negelectus* appear to form monogamous relationships and live in small family units. Tsingalia and Rowell

(1984) argue that one-male social units may not be the basic breeding units.

D. Multi-Male/Multi-Female Groups

Multi-male polygamous structures are probably the most familiar to people working with primates. The most commonly used laboratory species of monkeys, *Macaca* and *Saimiri,* live in large, usually multi-male, multi-female social groups. These groups are typically maintained around a central set of female matriarchies that act as the nucleus of the group. Females born into groups usually stay and remain attached to their matriarchy throughout their life. In rhesus monkeys the dominance rank of a female is related to that of her mother and sisters. Matriarchs tend to dominate the females born into their family. As younger daughters and granddaughters are born they are supported by the higher ranking matriarch even against related animals. This leads to an inverse relationship between the birth order and dominance rank; younger females in a matriarchy will tend to dominate older sisters. Although *Saimiri* also are usually described as multi-male, multi-female units in some subspecies, the adult males are peripheral to the female-dominated social structure except during the breeding season (Baldwin and Baldwin, 1981; Coe and Rosenblum, 1974). Field studies have shown that female relationships may vary between the Costa Rican squirrel monkeys and South American species (Boinski and Mitchell, 1991; Section V,B,2).

At puberty, males, females, or both may leave their natal group. Male rhesus monkeys typically share the dominance rank of their matriarchy until puberty. Shortly after puberty most rhesus males will leave the group and either directly join another group or spend some time alone before they join their next group. Males may leave and join different groups several times during their lives. The mechanisms involved in stimulating male transfer are unknown but there is little evidence that they are forced out. Once past puberty, male rank is influenced by many factors, but it is mostly highly correlated with tenure within the group, regardless of whether it is their natal group or a new group.

E. Fission–Fusion Communities

Chimpanzees (*Pan*) (Hiraiwa-Hasegawa *et al.,* 1984; Reynolds and Reynolds, 1965) and New World spider monkeys (*Ateles*) (Fedigan and Baxter, 1984) both live in social structures that resemble loose networks of individuals interacting together as social animals. These have been labeled as communities. *Ateles* groups form large sleeping groups that break apart for foraging during the day. The structure of the foraging groups is variable but the most frequent grouping is a female and offspring with an adult male. Chimpanzee groups or communities can range from 30 or less to over 100 animals. Subgroups can range in size from one to the total group, with groups of one to six being the most frequent seen. Males may be the most social among the chimpanzees with females spending more time alone or with just their offspring.

Variations on this theme are seen among hymadryas baboons (*P. hymadryas*) (Kummer, 1968; Sigg *et al.,* 1982) and *Theropithecus* (gelada baboons) (Kawai *et al.,* 1983). These species are typically referred to as one-male breeding units; however, these units seem to coalesce into larger social groups that travel and sleep together. The mechanism for maintaining the breeding units is entirely different between the two species. Hymadryas baboon males actively herd females and will retrieve those that have strayed too far. The males begin this behavior as juveniles by kidnapping a young female and actively training her. The primary bond is that between the male and each female. On the other hand, gelada females are the cohesive force behind their breeding units. They bond to each other. The male then joins the females, but does not herd them.

Large gatherings of gelada one-male units may be social aggregations, with little interaction between the units other than by female relatives. Hymadryas, however, have distinct levels of social organization above the one-male unit. Several units may constitute a clan that moves together over a long period of time. Clans consist of several related or familiar males. Several clans will come together to form a band and troops consist of several bands. However, the exact membership may vary with bands changing troops. Since troops primarily come together at scarce sleeping sites, the ability to mingle with other bands at the closest site each day would be an advantage.

V. TAXONOMIC GENERALITIES IN SOCIAL STRUCTURE AND ORGANIZATION

In recent years it has become apparent that many of our notions of how a species lives may be based on inadequate information. As the amount of field data continues to increase, it becomes evident just how much flexibility has been a major adaptive thrust within the order Primates. The following section provides a brief synopsis of primate social structures and social organizations for the four major divisions within the order Primates: prosimians, New World monkeys, Old World monkeys, and apes. The emphasis is on illustrating the differences and complexities within each division.

A. Prosimii: Prosimians

1. Lorisformes

Prosimians can be divided into the Lorisiformes and the Lemuriformes. Lorisiformes consist of the lorises of Asia and the galagos of Africa. These nocturnal animals sleep during much of the day and are characterized by a slow-moving quadrupedal climbing locomotion. They appear to be incapable of leaping

and usually move from tree to tree by bridging between branches. Their hunting technique relies on holding still. Galagos on the other hand are classified as vertical clingers and leapers. They are very active and agile animals.

Social groups are characterized by solitary animals or mothers and infants that have overlapping home ranges. Interactions between individuals are primarily restricted to the share of a common territory. Both males and females leave their mother at less than 1 year of age and move into their own territories. Females tend to move into territories adjacent to their mother's, if possible. Males may go through a stage where they are submissive of older, territorial males before they become territorial males themselves.

Bearder and Martin (1979) review three different classifications of social systems seen in the nocturnal lorisidae. Each type is characterized by differences in the amount of same sex contact that is tolerated. Galagos, for example, are categorized as a type 1 social system because of the considerable contact between mothers and daughters. Type 2 social systems include pottos and other lorises where the females do not interact much even along matrilineal lines. A type 3 includes those species in which the male and female territories coincide with little interaction with other animals.

2. Lemuriformes

Lemuriformes are restricted to the island of Madagascar off the coast of Africa. They are often divided into subgroups based on when they are awake and on their mode of transportation. Lemurs are diurnal semiterrestrial quadrupedal runners that eat fruits, insects, and flowers. Lepilemurs are nocturnal vertical clinging and leaping animals that feed on leaves and flowers. Indrii are diurnal (except the Avahi which are nocturnal) vertical clingers and leapers that eat a variety of things, including leaves, fruits, flowers, and seeds. Daubentoniidae live on the east coast of Madagascar in the rain forest. They have a well-developed olfactory lobe, large ears, ever-growing incisors, and a middle finger modified to form a thin probe, adaptations to eating insect larvae hidden under the bark of trees.

Cheirogaleidae are nocturnal animals that eat nectars, fruits, insects, and gums. These species live in the dry western forest of Madagascar (Charles-Dominique *et al.*, 1980). Winters are so harsh there that some species hibernate for as much as 6 to 7 months. Most of the nocturnal lemuriformes are solitary and show social arrangements similar to the lorisiforms, distinguished by overlapping territories. One species of mouse lemur (*Microcebus*) shows a type of social organization with male territories overlapping several females. Males visit and interact with the females whereas the females interact with neighbors, possibly grooming and sleeping with each other.

While the indri is thought to be monogamous (Pollock, 1979), more characteristic of the diurnal lemur species are multi-male/multi-female social groups (Taylor and Sussman, 1985). Data seem to support the idea that the females form stable groups and the males transfer between the groups. The exact relationships between the groups of lemurs appear hard to specify. Males seem to transfer freely in some cases and are harassed in others. The onset of the mating season leads to heightened intermale aggression and permanent changes in dominance relationships. However, female choice appears to be more important than dominance rank in determining access to estrous females.

B. Ceboidea: New World Monkeys

1. Callithrichidae

New World monkeys fall into two general groupings: marmosets and tamarins (Callithrichidae), and the cebid monkeys (Cebidae). Callithrichidae can be separated from other New World monkeys based on several characteristics. Marmosets and tamarins retain claws for climbing and have diets that are more akin to those of prosimians than most other anthropoids.

Both tamarins and marmosets are diurnal, quadrupedal arboreal animals that tend to eat insects, small vertebrates, fruit, and gums. In general they are smaller than the cebids and they have an extra premolar. Perhaps the biggest difference is that over 80% of the births contain twins. Wild populations of callitrichids were usually seen in groups of 2 to 10 animals that were assumed to be a breeding pair and several generations of offspring that served the group by helping to raise the twins. Good breeding in captive groups housed this way supported the idea that this was true. More recent field data have shown a high degree of variability in the callitrichid mating system (Sussman and Garber, 1987; Ferrari and Lopes-Ferrari, 1989). About 61% of the time these wild social groups contain only one breeding female and multiple adult, breeding males. The males in these groups do help with the rearing of the infants.

Rothe and Koenig (1991), on the other hand, have surveyed a number of captive breeding colonies of *Callithrix jacchus*. They suggest that deviation from monogamy may be rare in these situations and that the common marmoset generally follows a monogamous breeding strategy. This suggests that callitrichids show a great deal of flexibility in their social structures. Different species within this group may be very adaptable.

2. Cebidae

a. PITHECIINES. The Pitheciines consist of *Aotus, Callicebus, Pithecia, Chiropotes,* and *Cacajao*. Species in this group range in size from around 1 kg (*Aotus* and *Callicebus*) to approximately 4 kg for the *Chiropotes* and *Cacajao*. This group includes several genuses that are reported to be monogamous: *Aotus, Callicebus,* and *Pithecia* (Aquino and Encarnacion, 1986; Wright, 1981; Kinzey, 1981). These animals organize themselves into family units that usually consist of one breeding pair and several litters of offspring. Group sizes are usually less

than six. *Aotus* and *Callicebus* have extensive paternal involvement with the infants. The father generally carries the infants up to 80–90% of the time. Enough is known about *Aotus* and *Callicebus* to suggest that the juveniles are forced out of the group.

These species exercise fairly exclusive use of their territory, although it is controlled differently among the different species (Mason, 1968; Waser and Wiley, 1979). *Callicebus* are generally aggressive at the boundaries of their territories, although other species of *Callicebus* apparently use a more passive defense, moving away from any conspecifics (Kinzey and Robinson, 1983). Aotus tend to defend the trees when there is enough light to see clearly the other party (Wright, 1981).

Both *Chiropotes* and *Cacajao* are seen in large groups of 30 or so. *Cacajao* has been reported in large aggregations of animals; however, there is no good data on interanimal relationships (Fontaine, 1981). *Chiropotes albinasus* have been studied and appear to form pair units within a larger group. Population censuses have found the adult males and females in roughly equal numbers. Within their home range the adults can be seen foraging together and moving together in unison. Juveniles move about as a subgroup within the larger group.

b. ALOUATINAE. Alouatinae are widely distributed from southern Mexico to north Argentina. They are usually divided into six species that are essentially allopatric over the entire species range. Alouatinae are clearly vegetarians that concentrate on young tender leaves and green fruit. Troop sizes average around 10–20 individuals, with 2 to 3 males and 4 to 10 females.

Howlers have an age-graded male structure, as opposed to single-male and multi-male. One dominate male usually does the majority of mating (Eisenberg, 1979). Other males, thought to be the dominate male's sons, are tolerated as they mature. Evidence exists in some species that inter-male aggression is high.

Maturing individuals of both sexes emigrate from howler troops. The red howler emigrating females meet with males and form new groups. There is a constant splintering and reformation of groups. Mantled howler females move into established groups.

c. CEBINAE. Squirrel monkeys and cebus live in multi-male, multi-female social groups. Squirrel monkey group sizes can range up to 100 individuals, with an average group size for both genuses ranging between 10 and 30 animals (Baldwin, 1971; Baldwin and Baldwin, 1981; Terborgh, 1983). Squirrel monkeys have a polygamous mating system with high male aggression during the breeding season. Some subspecies express a sexual segregation during the nonbreeding season (Coe and Rosenblum, 1974). Males are excluded from the female juvenile group and travel together as a satellite group. Most affiliative behavior is seen between females and consists of moving together and sitting huddled together (Baldwin and Baldwin, 1981). Vocalizations appear to be a key element in the cohesion between squirrel monkey females (Boinski, 1991). As females in the wild move apart to feed there is an increase in the amount of calling. In the dense tropical canopy in which this animal lives this is an effective way of maintaining contact.

The stable of ranks among the squirrel monkeys seems to vary with the species (Mitchell *et al.*, 1991). The Panamanian (*Saimiri osteridi*) cannot be ranked consistently whereas *Saimiri boliviensis* maintain stable rank relationships that are consistent over time (L. E. Williams, personal observation, 1985). Male and female hierarchies are separate and the relative dominance status changes with the breeding season. During the nonbreeding season, females are generally dominant over males; however, males can supplant females during the breeding season.

Cebus maintain age-graded male structures similar to that seen in the howlers. There is one dominate male that usually is the only one to mate. The dominate male is not necessarily the most aggressive animal. In *Cebus olivaceus* it is usually the dominant female that is the most aggressive (Robinson, 1981). Dominance rankings are linear and rank reversal is rare (Izawa, 1980). Dominance status affects the spatial organization within *Cebus apella* groups (Janson, 1990). The feeding position of an individual is related to the amount of aggression it receives from the dominant male.

Grooming is very important and is usually directed toward the dominant male or female (Robinson and Janson, 1986). Dominant animals may well receive twice the number of interactions when compared to lower ranking animals. Both males and females emigrate from natal groups; however, the mechanism behind these transfers is not well understood.

Alloparenting plays an important part in the socialization of both squirrel monkeys and cebus. Squirrel monkey infants may spend up to 30% of the time on an allomother (Baldwin and Baldwin, 1981; L. E. Williams, personal observation). Allomothers tend to be young adult animals or females that lost infants earlier in the year. Some infants spend more time on allomothers during their first month, and infant swapping has been verified genetically in the laboratory (L. E. Williams, personal observation).

Although there is no allopaternal behavior reported in *Saimiri, C. apella* mothers regularly leave their infants with the dominate male (Robinson and Janson, 1986). Older infants are carried by same sex adults for extended travel periods.

d. ATELINAE. Atelinae consist of *Lagothrix,* the woolly monkey, which is widely distributed throughout South American forests; *Ateles,* the spider monkey, which is distributed in four allopatric species from Central America to the Pacific coasts of Columbia and Ecuador; and *Brachyteles,* the Murigui or woolly spider, which is restricted to the Atlantic coast of Brazil.

Spider monkeys form large groups that fragment into smaller feeding groups during the day, usually into groups of three or fewer (Symington, 1990; Chapman, 1990). This fission–fusion type of social structure is very similar to that seen in the chim-

panzee (*Pan troglodytes*). The mother–infant combination is the most frequent and stable subgroup. Male–male aggression is low. Estrus females actively choose their male partners so there is little competition among the males (Eisenberg, 1976). Males can be easily ranked in a dominance hierarchy; females, however, show signs of a rank but rates of aggression are generally very low. Males are the most affiliative individuals, with more interactions directed at both males and females (Fedigan and Baxter, 1984). Because females transfer from troop to troop, the males may be more highly related to one another. Females will often visit other troops carrying a newborn (Symington, 1987).

C. Cercopithecoidea: Old World Monkeys

1. Colobinae

Group sizes and composition vary greatly among the colobinae (Struhsaker and Leland, 1985). The majority of the species live in groups of around 10 animals, containing one adult male and multiple adult females. The multi-male species live in larger groups of between 15 to 80 animals depending on the seasonality of the habitat. Larger groups have been reported, over 100 *Colobus angolensis* in Rwanda and several hundred *Rhinopithecus* (golden snub-nosed monkey), but these may have been aggregations of several groups instead of one large social group. Yeager (1990, 1991) has reported that *Nasalis* show a system similar to the gelada baboon described in Section IV,C,2,c. One-male groups coalesce around sleeping sites but separate during the day.

Two cases of monogamy have been reported; in both cases the species live on the island of Siberut off of Sumatra (Watanabe, 1981). *Presbytes potenziani* show both the dueting and mutual display characteristics of true monogamous species. The other species, *Nasalis concolor*, does not show these traits and may be polygynous in higher densities.

All-male groups are rare and have been described mainly in *Prebytis* (Langurs) (Hrdy, 1977; Gurmaya, 1986) and *Nasalis* (Proboscis) (Yeager, 1991). These groups regularly go on to the territoriality of other groups but do not defend any territory of their own. The fact that all-male groups are seen in some species suggests that the males transfer. Most of the male–male interactions are aggressive. In those species where multi-males are seen, there is usually a clear dominance hierarchy.

Females appear tolerant of each other, and dominance hierarchies are hard to spot because of low levels of aggressive interactions (Hrdy, 1977). However, in some langur species dominance among the females is strong and reversals are frequent. Grooming patterns are typical for Old World Monkeys, with females tending to groom both males and females. *Nasalis* females direct most of their attention toward either their offspring or other females in the group (Yeager, 1991).

Males tend to groom very little and it is usually directed toward adult females. Intergroup relationships are mostly aggressive, particularly when it comes to males. Territoriality is seen in most populations of langurs.

A regular exception to the general colobus rule is the red colobus (Struhsaker and Leland, 1977). Females transfer at the time of puberty. Although some males leave their natal troop, most stay. Aggression is rarely seen, even though the groups typically have a multi-male structure. Females groom each other less than expected, whereas males groom males more than expected and females less than expected. Most populations of red colobus show no evidence of territorial defense. Females transfer troops and males stay in their natal group. This means that the males are more highly related and familiar with one another. They might be expected to interact at a higher rate than groups where the males transfer out.

2. Cercopithecoidea

a. GUENONS. The guenons typically inhabit forests of west and central Africa. A great deal of sympatric overlap appears to exist between species, with several species in the same area using the same resources, participating in activities together. *Erythrocebus patas* is the only nonforest, terrestrial monkey in the group and occupies savannas and tall grasslands.

All the species in this group have social groups of about 10–40 animals. Males leave their natal groups at puberty and live part of their life as solitary animals or as part of a new social group. All-male groups have only been seen in the patas. Some populations of *Cercopithecus neglectus* (DeBrazza's monkey) may live in small family groups, with one adult male and female plus young (Gautier-Hion and Gautier, 1978). This may be a function of population density as some reports have higher group sizes with more than one adult female (Brennan, 1985; Leutenegger and Lubach, 1987).

Some species set up clear territorial boundaries and show territorial defense and exclusive use of parts of the home range. This varies from aggressive defense in some of the forest dwellers to avoidance in patas. In general the larger the home range, the harder it is to defend and it less likely that one will see active defense. Females tend to be more aggressive in territorial defense than males (Chism *et al.*, 1984).

Social interactions within guenon groups are not very clearly understood. Guenons seem to interact seldom and dominance hierarchies are hard to discern because of the low interaction rates. Females and juveniles interact the most, suggesting that mother–offspring ties are strong. Among the patas, kinship is thought to be the major organizing feature that affects all affiliative interactions (Loy and Harnois, 1988). Allomaternal care is extensive in a number of the guenons, probably most so in the patas (Chism *et al.*, 1984).

While guenon social groups are generally discussed as one-male, multi-female social units, a common event in some species is the rapid, sequential turnover of males in social groups during certain mating periods. Tenures of these males may range from 1 day to several months. Evidence that the one-male,

harem style mating structure is the true basic unit comes from group censuses that show there is usually only one adult male present in the social group. Males in groups are generally intolerant of other males. Strange males are threatened and chased away (Cords, 1986).

b. MACACA, CERCOCEBUS, CERCOPITHECUS AETHIOPS, AND MIOPITHECUS. All the species in this group show the same basic social structure, multi-male/multi-female groups with a sex ratio skewed toward the females. The groups typically move as an integrated unit and do not regularly split up into subgroups as is described for the hymadryas and gelada baboons in Section IV,C,2,c. Talapoins may be the exception (Rowell and Dixson, 1975). Studies have suggested that they tend to move in distinct male–female subgroups; however, the subgroups remain together over time. This fact, together with their diet of insects and high daily travel rates, has led some to suggest an evolutionary convergence between the Old World talapoins and the New World squirrel monkeys.

Females do not typically emigrate and form the core, social nucleus of the groups. Close bonds are maintained within the matriarchies. Stable, linear, dominance hierarchies among the females are the norm. Rank is typically passed on to the daughters in an inverse fashion, so that the youngest daughters tend to rank higher than their older siblings (Berman, 1980).

These species tend to gather in multi-male groups. Therefore male–male competition is within the group, not between group males and single (or all male groups) as in the guenons. There is a higher tolerance for males than among guenons; however, most of the interactions between males are still aggressive or dominance oriented. Even when they are cooperating in aggression toward a third male, there is little positive interaction other than running beside each other or mounting. Male–male grooming does occur at a very low rate, mostly during the nonbreeding season.

Males usually have a clearly defined dominance hierarchy that is not as stable as that seen between females. Males lose their matriarchy rank at puberty. Most emigrate at this time, but not all. The causes of male transfer are not known. Males may travel alone for a while but eventually attach themselves to another social group. Émigré males may sit peripherally and play with juveniles or try to attach themselves to a peripheral, low-ranking matriarchy. This helps them establish access to the social group.

c. PAPIO. There is as much variation within the group of species called baboons as within the whole group of Old World monkeys. Baboons range throughout most of Africa, from the high deserts of Ethiopia through the forests of central Africa, into the savannahs of west and south Africa. Although taxonomies have differed over the years, four groups of animals are generally thought to fall within the baboon collection: hymadryas, gelada, drills and mandrills, and savannah baboons. Each lives in a different type of habitat and displays very different social structures.

The social organization of the hymadryas, gelada, mandrills, and drills is based on the one-male unit. The savannah baboons typically live in multi-male/multi-female social groups. However, some of these subspecies have a tendency to move in one-male units when population densities and environmental conditions make it advantageous. Even though they share the one-male unit structure, the mechanisms for each are very different.

Hymadryas social structures are often divided into three distinct levels (Kummer, 1971; Abegglen, 1984). Certain one-male units associate together in what are called "clans." These clans move together more frequently during travel and foraging and may consist of males that are closely related. Several clans, possibly with single males, form very stable social groups called bands. Social interactions are directed almost exclusively toward "band" members. "Troops" consist of several bands which have come together at sleeping sites or at other scarce resources.

Juvenile and subadult males have several strategies for starting their own one-male units. The males that remain in their natal band may adopt young females or at least establish a relationship with them that removes them from their natal unit. Males may also attach themselves, as followers, to an established one-male unit. As the older male ages the younger male assumes more and more control.

The male is the focus of social attention in the hymadryas one-male unit (Sigg and Stolba, 1981). He receives the most grooming. He will actively herd female unit members by chasing them and using neck bites to punish females that stray too far. Female bonds are strongest with the band. Females may move to a different one-male unit through contacts with familiar animals.

Gelada one-male units form clusters of closely related individuals called bands (Kawai *et al.*, 1983). Geladas are different from hymadryas in that band membership is not stable; one-male units may visit other bands or may move off alone. Single males associate in all-male groups that may or may not travel with a band. Herds are formed of bands that come together for feeding or access to other restricted resources. Social interactions between the bands are limited even when they are spatially close.

Among the geladas the females form the main bond within the one-male units. Females within a unit are thought to be closely related as there is little movement between units. Males are peripheral to the majority of social interactions with the one-male unit and may associate with only a few females regularly.

Males may start out as a follower, similar to the hymadryas, and attack or break off from an old leader or may take over a unit during a raid by an all-male group. Older males may stay with a one-male unit as a submissive male. Male–male social interactions are rare among the geladas (Dunbar and Dunbar, 1975). Male herding of females, although present in the geladas, is very different than that seen in the hymadryas baboons. The males use submissive gestures and prod the female back to

the unit. If they become too aggressive, the females will chase and attack the male (Dunbar, 1983).

Drills and mandrills are also thought to live in one-male units. Although there is little field data, it has been suggested that these one-male units may come together during dry seasons to exploit resources. Frequent interunit and intraunit vocal exchanges suggest that long-term relationships may exist (Kudo, 1987). Adult males seem to live in either one-male units with females or alone; there are no all-male groups (Emory, 1975).

Data are lacking on the formation of social units in drills or mandrills. It has been suggested that given the existence of single males, the gelada takeover pattern may hold; that is, females staying together with males changing leadership of social units through attacks. Data from captive groups indicate that the mandrill and drill males are as peripheral to the social group as the gelada males, with most social interactions appearing to occur between the females.

Savannah baboons live in multi-male/multi-female social groups very different from the one-male units described earlier. This specie consists of several subspecies that live throughout the African savannah region: *cynocephalus* (yellow baboon), *ursinus* (chacma baboon), and *anubis* (olive baboon). Savannah baboons have been called generalized feeders, eating everything from grasses and flowers to insects and small mammals.

Group sizes range around 20–40 individuals with a female-to-male ratio skewed to a larger number of females in the group. Social groups typically move together as an integrated group and do not split into different subgroups on a regular basis as described earlier. Females usually remain in their natal group for all their lives. They form the stable social core of the group. Females usually have a linear style dominance hierarchy that is fairly stable (Altmann *et al.*, 1977).

These social groups contain more than one male and competition for access to estrus females occurs within the group. Males typically transfer groups around the age of puberty. Although the proximal causes of male transfer are not clearly understood, the immediate result is that the males within a group are less related to one another than the females. Male–male interactions are generally more aggressive than affiliative. There is a greeting response between males that is characterized by stereotypical vocalization, facial expressions, ritual mounting, and touches. While competitive factors do influence access to matings for males, tenure in the group (length of stay), alliances with females and matriarchies, and female choice are usually just as strong determinants of who mates with whom.

D. Hominidae: Lesser and Greater Apes

1. Hylobatidae

Hylobates are monogamous and territorial. Hylobate social life has been described as subdued (Leighton, 1986). Group size ranges from the mating pair to the pair plus three offspring. Gibbons spend a lot of time sitting together with very little social interaction. Males and females are approximately the same size and there is no consistent dominance relationship between the sexes; sometimes the male is dominant, sometimes the female. Perhaps since there is no variety in social partners there are no demands to maintain the complex social bonds seen in primates that live in groups with more than one member of a given sex.

Hylobates actively defend a territory. Routine spacing is maintained through loud morning song bouts and ritualized encounters with neighbors at range boundaries (Mitani, 1985b). Gibbons have species-specific, loud songs that can be heard at distances of up to 1 km (Raemaeker and Raemaeker, 1985). Males tend to sing in the predawn darkness. Unmated males will sing longer than mated males, whereas unmated females tend not to sing. Pair duets in the early morning can start spontaneously or as the result of neighbors calling. Most species sing duets daily.

Territorial defense is carried out by both sexes. Males are usually more aggressive. Defense aggression ranges from staring at the other male to actual contact. Females usually sing and add moral support.

2. Pongo

The primary social units for orangutans are solitary males and females with offspring. Sexual consortships may last up to a week. Dispersal occurs but has not been well documented. Females appear to move to territories adjacent to their mother's territory. Males may move around quite a lot, sometimes staying close by and other times moving far away.

The mating system involves a territorial structure similar to the galagos: smaller female territories overlapped by a larger male territory (Rodman, 1973). The fact that these are slow-moving animals and that they have large territories makes it difficult to defend exclusive access to a territory. Male territories probably overlap extensively and evidence suggests that mating may be promiscuous. Mating strategies vary with age. Subadult males may use force to make a female mate. These forced copulations are resisted more frequently by the females and are rarely successful. Subadults may hang around females for several days or until they are run off by an adult male (Mitani, 1985b).

Adult male patterns show two different patterns. Some adult males try to set up territories that incorporate several female territories. Other adult males appear to wander over greater areas, opportunistically mating as they encounter females. It is not clear whether one strategy or the other is more successful. Adult males do not interact much with each other and use loud calls to mediate spacing. Interactions among females are infrequent but are usually affiliative.

3. Gorilla

Gorillas live in relatively stable groups with both males and females that range between 12 and 40 animals. Around 60% of

the groups documented have only one adult male (Harcourt *et al.*, 1981). Both males and females emigrate from their natal group. Females usually transfer to another group or attach themselves to a single silver back but never range alone. Female transfer normally occurs when two groups come into proximity. Males tend to transfer out before they breed. They may join an all-male group or wander for years alone. Females may associate themselves with a single silver back (mature adult male). Displays or aggressive interactions when two males meet may attract females during encounters with social groups.

Social organization within the gorillas is centered around the silver back male (Harcourt, 1979). He is the dominate male and exercises a control role in most decisions of the social group. Group cohesion revolves around the relationships between the adult male and the adult females. Females with young offspring spend more time next to the silver back male. Immatures are strongly attached to the dominate male, they only spend more time with their mother. They seek him out and work to maintain proximity to the male.

4. Pan

Chimpanzee communities contain all age–sex classes and number from 20 to 100 individuals (Hiraiwa-Hasegawa *et al.*, 1984). Within these communities chimpanzees that associate together are called parties. These associations can last up to several days and vary in size from 1 to over 60, although parties of 6 or less accounted for over 80% of those counted (Goodall, 1968). Although any combination of age–sex classes may make up these parties, mother–infant pairs are the only stable parties. The mother–infant relationship lasts for several years beyond weaning and it is common to see older males and mothers traveling together. Older females and mothers do so to a much lesser extent. Unrelated females interact only infrequently. Another common party consists of mothers with infants traveling together.

Adult males are consistently more sociable than adult females. No long-term relationships appear to exist between unrelated males and females. Consortships, with extensive interactions, may last for a day or a week. Female chimpanzees transfer from community to community. Data suggest that they migrate before their first birth during a period of estrus (Nishida, 1979). Once they have given birth they tend not to transfer any more.

Bonobos also live in the community style social organization; however, mixed age–sex parties predominate the subgroups (Nishida and Hiraiwa-Hasegawa, 1986). Over 90% of the bonobo subgroups consisted of mixed parties compared to about a third of chimpanzee parties. Four types of groupings are typically seen: matrifocal, male bands, male singletons, and female singletons. Matrifocal parties include mothers and offspring, including adult males, and are relatively stable. Male bands consist of adult males of unknown relatedness. Male singletons are usually old and/or handicapped, whereas female singletons are nulliparous females who are presumed to be immigrants. Aggregations of matrifocal units form the usual bases for a party. Matrifocal units may also band together with a male band. Uehara (1988) reported that at least two communities used the same habitat without intermingling populations.

A male dominance hierarchy is linear as with the chimpanzees. Food sharing and grooming are most frequent between the sexes and between females, but not between males. Male bonobos interact less among themselves than chimpanzee males.

Bonobos seem to use sex in greeting and in the maintenance of affiliative bonds, not just around the time of ovulation (Savage-Rumbaugh and Wilkerson, 1978). Bonobos have many signs and gestures to signal readiness or willingness for sexual interactions. Aggressive behavior, on the other hand, is milder than for chimpanzees. There are fewer gestures and less complex interactions are seen. Bonobos have more stable parties than chimpanzees and show lower levels of competition. This might be related to the lusher environment the bonobos live in as there is less of a need for competition.

VI. METHODOLOGY USED IN STUDY OF BEHAVIOR

This section introduces research design and methodologies used in the study of primate behavior. It is included to assist those responsible for evaluating the proper care of animals and experimental protocols that require the use of animals. The methods used in the study of behavior are not unique to the topic but represent the natural extension of the general methods used in science to obtain data and test hypotheses.

A. Observation of Cause and Effect

In any scientific endeavor one begins by observation. "Observation" can mean reading the available literature and benefitting from the experiences that others have had because of their efforts. It also can mean watching or recording the subject of interest until one has seen enough or heard enough to note some regularities. When asking why one animal injures another or why one animal behaves in a certain way, look at the situations and try to find some common theme or variable in these situations. This type of logical search, for the answer to why particular behaviors occur in a particular situation more frequently than is attributable to chance, is the inductive process. It is a search for some event that regularly precedes the behavior and the use of statistical methods to infer a causal relationship in which the event is the proximal cause (stimulus or trigger) that elicits the behavior of interest.

B. Anecdotal Evidence

When a relationship is discovered between an independent and a dependent variable of interest, derived by inductive logic,

the tendency is to want to share it. This hypothesized relationship may be an extremely important insight that will profoundly alter thinking in the field or one that may have important practical application. Publishing inductive "findings" at this point may be ill advised. In published scientific papers, conclusions are supported by data and argue for a hypothesis by presenting evidence. However, the only data leading to an inductive conclusion are the observations that led to the formulation of the hypothesis.

Using the same data that generated a hypothesis to test the hypothesis leads to several types of errors. Applying statistical techniques to existing data ignores the fact that once an event has occurred, the probability of that event is 1.0. There is no way that one can show that the frequency of an event that has already occurred exceeds its probability of occurrence (1.0). For example, the probability associated with a family that already has five children, all of whom are girls, is 1.0, whereas the probability that the next five children born into a family will all be girls is 0.5 to the fifth power (32 chances in a 1000). The first family was not a random sample but is noteworthy because it was unusual. Given a very large number of families it can be expected that many will be skewed toward one sex or another even if the sex of each child is absolutely random.

Proving that a hypothesis does account for earlier observations is only a demonstration that inductive processes were sound. Applying statistical analyses to the original data is making a post hoc test to prove the induction already made. This in no way displays the value of the theory in predicting new data outcomes. New data must be collected to test the prediction. Postdictions which lead to an error of "affirming the consequent" only prove that our explanation is a satisfactory explanation for what is already known. The value of science is in predicting that which is not already known. In extreme cases it is possible to account for a single observation, an anecdote, by inventing a plausible theory and then using the anecdote as evidence for the theory. A collection of old anecdotes does no better.

An unusual event may call itself to our attention. An unusual combination may seem noteworthy, but these are anecdotes and may need no more explanation than that enough observations will uncover rare combinations. If this combination, however, seems lawful and we can predict the circumstances under which it occurs, then it is noteworthy and deserves proper testing and verification.

C. Hypothesis Testing

The next step in properly testing a theory is to use deductive logic to produce a specific hypothesis that predicts how our independent variable will effect the dependent variable. These variables may be yes–no measures (male or female) or may involve points along a continuous scale like a number of behavioral responses. If the hypothesis is that males are inherently more aggressive than females and that chases are a good measure of aggressive behavior, the experiment is to measure chasing behavior in a population that is male and one that is female. The specific prediction is that the males will chase animals more often than will the females. The null hypothesis is that being male will not influence how often an individual will chase others or that males will not chase others more than will females. Statistical tests are designed to test the null hypothesis that the independent variable did not produce the changes predicted in the dependent variable, i.e., that the two variables are random with respect to one another.

Since statistical tests only state the probability of the null hypothesis being true, what level of confidence (the reciprocal of the probability of the null hypothesis being true) is required before the null hypothesis can be rejected, that the data do not indicate the predicted relationship between the independent and dependent variable? By convention, many people work at the 95% confidence limit saying that the null hypothesis will be rejected if there is a 95% confidence level that it is false or that there is less than 1 chance in 20 that it is true. However, there is no magic number. Confidence limits should be set after evaluating the consequences of failing in order to reject the null hypothesis or falsely rejecting the null hypothesis. (Few people would fly if there was only a 1 in 20 chance of arriving at their destination safely.)

Once the confidence limit has been selected it becomes a decision point. If a 95% confidence level must be reached before the null hypothesis is rejected, then only if a statistical test indicated that the probability of obtaining a given set of data by chance was less than 0.05 will the null hypothesis actually be rejected. Probabilities are not correlations and a probability value of 0.001 does not mean that the influence of the independent variable on the dependent variable is "stronger" than if the probability value were 0.05. If the influences of the independent variable are constant, the p value of a test will decrease simply as a function of sample size. Seven thousand out of 10,000 will produce a p value much less than 7 out of 10, although the bias is still 70%.

Similarly, if 0.5 is the maximum probability that will be tolerated in rejecting the null hypothesis, then a finding of 0.51 does not meet the preselected criteria and the null hypothesis cannot be rejected. Claiming that it "almost made it" does not change the fact that the findings are nonsignificant. Similarly, since probabilities are not correlations, there was not a "trend" toward significance. This is an arbitrary decision point, and 2 out of 3 heads does not mean that if we continue we will observe 200 out of 300 heads when tossing the same coin.

If the difference in chasing scores in two groups is greater than could be attributed to chance (random variation in measures, sampling of subjects, etc.), it may still be for reasons other than males being inherently more aggressive than females. Perhaps older animals are more aggressive and all our males were older than our females. This variable, age, is a confounding variable that may account for obtaining the data we predicted.

The hypothesis may have predicted correctly, but for the wrong reason. Controlling the selection of subjects to rule out the possibility of age can be done in two ways. One is to fix age so that all subjects in both groups are the same age. The other technique is to randomize age so that subjects are chosen for the two groups randomly from a population containing all ages. Randomizing assumes that the groups will not be accidentally biased by the age factor. Fixing the age factor may be more comforting but may mean that the data and conclusions are limited to a subset of males and females of a particular age. Matching ages in the male and female groups may be a good compromise so that each subject in one group is matched with another in the other group on all variables except the one of interest, in this case sex. Certain unavoidable confounds may persist; for example, males may, by virtue of their sex, be larger than females or have different hormonal levels. However, these confounds are so intimately related to sex that it is possible to argue that male versus female implies differences in hormones and size and that these variables are part of the sex variable and therefore need no control.

Having controlled for age, colleagues may next ask if health, time in the laboratory, birth order effects, the month of birth of our subjects, or even the time of day that they were born had been controlled. Indeed, the number of alternative confounding variables is limited only by imagination. Investigators need not control for all possible confounds, but only for the plausible ones. However, what alternative explanations are as plausible as the theory we are testing? That is a subjective decision and it is plausible that a valid alternative may be rejected as totally implausible in one laboratory but be the bases of a large research effort in another.

No hypothesis can truly ever be proven. Evidence is amassed by repeatedly making and verifying predictions. If the theory makes more accurate predictions than any competing theory, it may be extremely useful, but it will be virtually impossible to prove it is true. Similarly, failure to demonstrate that males chase more often than do females does prove the null hypothesis. Perhaps the theory is correct but chasing (the measure of the dependent variable) was poorly measured. Perhaps sex was misidentified in some of our subjects. Any experiment, done badly enough, will fail to reject the null hypothesis.

A failure to reject the null, or negative evidence, can be useful if the experiment is restated to say that the bias is less than 90% (or any preselected number) and the null hypothesis therefore is that it is greater than or equal to 90%. If the data do not support the null of a bias greater than or equal to 90%, that hypothesis is rejected in favor of the hypothesis that the bias is less than 90%. This kind of formulation never proves that there is no influence but can be used as evidence that the influence is less than a certain amount. "Proof" of course is a probability statement. A probability level of <0.1 would mean that there was a 99% chance that these results did not occur by chance.

Normal distributions imply that all events can happen, but that some events are very rare. Four-leaf clovers may be rare, but if one looks through enough clovers they will find several examples. Once they are in a collection the probability of finding a four-leaf clover in the collection of four-leaf clovers is very high. Any penny will show 10 "heads" in a row if it is tossed enough times.

Experimental methods ultimately rely on measurement and definition. Conclusions will be probability statements and tests require quantitative data, even if only on a yes–no scale. The independent variable may be manipulated by its presence or absence, e.g., a diet with an additive or a diet without an additive. It may be manipulated on a graded scale, as in grams per kilogram of diet or by percent of diet or by absolute amount. Independent variables can be useful even if they cannot be directly manipulated as long as they can be measured. Experiments can be performed when the independent variable is morning or afternoon, winter and summer, or pre- and postpubertal. These times are not "manipulated," but can be measured and assigned as a treatment to subjects.

When using an independent variable, like season, to see if a dependent variable, like aggressions, varies with the season, there is sometimes a temptation to measure all possible behavioral differences as a function of season; the influence of a host of independent variables on a dependent variable like aggressive behavior. The more things that are measured, the greater the possibility that a difference will be found due to sampling error. If enough coins are tossed 10 times, each eventually will show 10 heads in a row, although it is truly not biased. This is the same problem as noted in tossing the same coin long enough.

D. Behavioral Data Collection

The actual measurements themselves can be done under a variety of circumstances and using multiple techniques. A sample should be selected that will represent the population from which it was drawn. It is seldom possible to measure every individual in a population; the hope is to obtain an unbiased representative sample. Data collection can now proceed under the specified conditions.

When collecting data on behavior, it is possible to focus on acts or responses that are essentially instantaneous. A slap may have finite duration but it is very brief and the amount of variation in the duration of slaps is very small. If slaps are distributed in time randomly with respect to one another, then each slap is an independent measure of slapping. These data are distinct acts and are reported as a frequency or the frequency is divided by the observation time and a rate of some sort is reported, like slaps per hour. Other types of responses, such as sitting in contact, are behavioral states and a measure of frequency would be difficult (unless the frequency of first making contact when sitting next to another is of interest). Sitting in contact can last a long time, it may be briefly interrupted as individuals adjust their positions, and the duration of a bout of

sitting together may be extremely variable. Instead of a frequency, a duration measure may be desired. It may be expressed as minutes per hour in contact or percent of time in contact or any similar measure of duration.

Some acts have discrete times of onset and cessation and are relatively independent of one another. Questions concerning how long the average chase lasts in a group can be answered by recording both frequency and duration and dividing the latter by the times.

On the other hand, some acts have little duration but are clustered in bouts, that is each act is not independent of the occurrence of the previous act. Individuals engaged in play frequently pause and resume the activity. A frequency is hard to define and the duration can either include or exclude the brief pauses. A pair of individuals may slap one another in a fight repeatedly and slapping may therefore occur in little bursts or bouts. Fights may vary in duration and the number of slaps delivered may vary from fight to fight. The duration of slapping may be impossible to assess and be perhaps meaningless as a measure of aggression. The frequency of hand contacts also may be a meaningless measure of aggression. In such a case it is the frequency and duration of fights that should be measured.

Since fights do not take a single form and often lack a single motor description, the question then becomes one of operationally defining "a fight." One possibility is to calculate neither a frequency nor a duration but a probability measure. Here we could ask what the probability would be that we would see one or more slaps if we watched our subject for N minutes. If the probability is higher in one group than in another, then we may say that we are more likely to see slapping in that group than the other group despite the true frequencies and durations involved.

1. One–Zero Sampling

Jeanne Altmann (1974) has reviewed all the data collection schemes in common use to obtain frequency and duration measures. She has argued that one–zero sampling provides data on neither frequencies nor durations but an indeterminate index of both. She dismisses one–zero sampling as useless in measuring frequencies and durations, but it is possible to use this technique (was the behavior seen during the preset interval of observation: yes or no) to calculate probabilities. Probabilities are every bit as useful a quantitative measure as frequency or duration and the research investigator must choose among techniques according to the properties of the variables of interest.

2. Instantaneous Scans

Doing an instantaneous scan of a subject to assess involvement in a behavior state, like sleeping, requires that a "snapshot" in time be taken of the subject at preselected times. A percentage of time spent asleep is calculated by dividing the number of snapshots in which the subject is seen asleep by the total number of snapshots. These point samples of the animals behavioral state can be extrapolated to an estimate of the amount of time that the subject spends sleeping. Two caveats are in order. First, the snapshot cannot be truly instantaneous. It must be long enough to decide that the subject was sleeping and did not just happen to blink or close its eyes. Second, because samples taken at 1-min intervals are separated by far less than the average duration of a sleeping period, they are not independent of one another. One hundred samples of 1-min intervals during a 2-hr nap may suggest that the subject sleeps 100% of the time. Instantaneous scans must be performed at intervals that exceed the duration of most instances of the behavior of interest to ensure independence of sampling.

3. *Ad libitum*

Since no one can watch everywhere at once, Altmann criticizes *ad libitum* data collection, on a large battery of possible responses for multiple subjects, as inherently inaccurate. This type of data collection will obviously tend to include more data on readily visible individuals and more examples of prominent attention-catching behavior, like noisy fights at the expense of quieter, more subtle, and more rapid responses like a glance or yawn. Altmann states that if the observer focuses on a single, readily observable response, it is possible to score all instances of the behavior that occur among a group of individuals, all of whom are in view. This method of all-occurrences scan requires that the responses (1) be readily recognized (perhaps because it has a "loud" activity component); (2) have a low to moderate frequency, such that it is unlikely that multiple episodes will start simultaneously; (3) have sufficient duration such that the event is unlikely to be missed as the members of a group are scanned; and (4) be just as observable in all possible subjects. These conditions can be met under many circumstances, but when observers try to use this technique for less visible actions or try to increase the number of actions being scored during an all-occurrence scan, this becomes equivalent to *ad libitum* scoring.

Ad libitum sampling may seem to have no justification, but it is still useful in obtaining pilot data, in searching for the existence of unusual patterns, and sometimes for detecting the directionality of social behavior independent of their frequencies and durations. The directionality of agnostic behavior in groups with well-established dominance relationships may be all that is needed to document dominance relationships. True frequencies, durations, and probabilities may add little additional data concerning which of two individuals is dominant.

4. Focal Animal Sampling

Focal animal techniques allow the observer to record the onset (and cessation) of states and the time of occurrence of an action with small durations and to do so for a large number of behavioral categories. Sequences also can be preserved using

this technique. There is a cost. Data involving animals other than the focal animal and not directed toward the focal animal will be lost. In a group of 100 individuals it will take 100 hr to obtain 1 hr of data for the group. If the data represent dyadic social interactions, each hour actually represents 1 hr on the focal plus 1/99 of an hour on each of the possible interacting animals. If the observer records the behavior of all animals for 1 hr each, there will be 2 hr of data on the group. Even so, it requires a lot of time to collect as much data as could be collected using instantaneous scans or all occurrence scan techniques. The advantage, of course, is that the observer gathers information on the frequencies, durations, and sequences for many more behavioral categories than the other techniques can provide.

5. Sequential Analysis

Sequence data may prove especially difficult to analyses. One can record all items in an ethogram during animal scoring and then search for the frequencies of combinations. These combinations are assessed as significant if they occur more often than the individual frequencies of the two items would predict. Such an exhaustive analysis may prove exhausting, even if large computers are in use, when one considers an ethogram of 100 items taken N at a time.

An alternative is to predetermine the combinations or sequences of interest and to record these sequences as a single instance. A trivial example is chasing which usually means one animal flees while another runs toward it. More complex sequences also can be identified. Asking whether chasing occurs seems superfluous but asking if chasing while vocalizing is more or less common than silent chases or more or less likely than hitting another and then running away may allow for a more discrete analysis of the behavioral interactions.

The recognition of sequences depends on the ability to recognize regularities in the co-occurrence of two behavioral items during the collection of pilot data. The alternative, a systematic analysis of all combinations, may reveal additional sequences, but the cost and effort may be prohibitive.

VII. APPLIED BEHAVIORAL ANALYSIS

In studies of agonistic behavior, aiding, or other third party interference in dyadic encounters or sequences like affiliation such as those following an agonistic encounter or between a mother and infant, our data collection techniques focus on whether the pattern exists. Data collection then is not random but is designed to answer specific questions about the animals or situations of interest. The next two sections discuss two important areas of captive primate management; aggression interactions between animals housed together and maternal care of offspring. The focus in each is on how behavioral analysis has answered specific questions and on how to apply the techniques described earlier to new problems.

A. Aggression and Group Formation

Any time two or more animals come together there is the possibility of violent conflict. Two similar individuals with similar needs may both want the same thing simultaneously when that "thing" is in limited supply or situated such that only one can gain access to it at a time. The two individuals then compete for access to the resource and may do so either by scramble competition, racing to get there first or acquire the resource first, or by conflict competition where one or both actively attempt to preclude the other's access to the resource. This may take the form of pushing, threatening, or actively attacking and attempting to drive the rival away.

Although competition is not the only source of aggression, it is so common that when we see aggression by a singly housed animal directed toward itself this is quickly labeled nonfunctional or pathological aggression. Any time there is no apparent cause or function such aggression is deemed to be pathological. Of course some of it may be displaced behavior or behavior redirected toward an available partner; some aggression may reflect irritability as in pain-induced aggression; some may be defensive on the part of a frightened individual; and some may be caused by abnormal endocrine, chemical, or neural stimuli. Although it should be possible to identify abnormalities responsible for heightened levels of aggression, this is often frustrated by the "normal" aggression seen in socially housed animals directed toward a cage mate.

Without question there are long-term benefits to social housing primates that normally live socially. The aggression that they direct toward one another when they are provided with social companionship seems particularly perverse. However, it is not unlawful nor is it without natural coping mechanisms that can be exploited to reduce the magnitude and frequency of the problem. It is not possible to eliminate aggression in socially housed primates, but it can be controlled to some extent such that it is manageable.

There are no magic solutions, no drugs, or treatments that will control aggression and interfere with no other bodily functions. Active policing of social interactions may work, but at some point we have to leave the animals alone; active policing may only teach the animals to refrain from active aggressive displays until the observer leaves. This may actually exacerbate the problem in that it may not be possible to identify a potential problem of incompatibility if all animals sit quietly in their corners while being observed and wait until they are alone for resumption of hostilities.

Distracting animals so that they are fully occupied in other activities and have little time for fighting may seem like a constructive approach. Anything that is positively attractive has the potential to excite competition and any noxious stimuli that

animals are motivated to avoid may stimulate competition for the appropriate escape or redirected aggression toward the partner.

Providing the partners with physical structures that can be used to hide behind or in, or that permit self-defense through a single narrow entrance, may have some short-term benefits. Since permanent escape is not possible, the conflict must eventually be resolved. An effective means of self-defense may seem "fair" but may prolong a conflict until one or the other can finally gain the upper hand and resolve the conflict by definitively defeating the opponent.

Aggression is naturally controlled by one individual definitively defeating another. Once the inequality has been established and agonistic behavior is totally asymmetrical in a pair, a dominance relationship has been established and the dominant individual rarely needs to take advantage of subordinates by attacking them until they are dead. Dominant animals will reinforce their dominance with milder and milder forms of aggression and become satisfied with a grimace, a gesture, a supplantation, or a simple avoidance or aversion of gaze as a reaffirmation of a stable dominance relationship. Only when signals affirming the relationship are absent will a normally dominant animal escalate aggression against an established subordinate.

Dominance relationships, once established, order agonistic exchanges in competitive conflicts, reducing the duration and intensity of conflicts. When two individuals with an established dominance relationship come into a competitive situation and a conflict is provoked, the first aggressive signal of the dominant is typically answered by submission from the subordinate. The subordinate generally withdraws and the dominant uses the resource. Only after the dominant is finished will the subordinate gain access to the resource. Such exchanges may seem unfair, but the dominant monkey seldom hordes a resource after it is satisfied. As long as there is an abundant supply of the resource, dominance merely results in orderly turn taking. One result of this, however, is that reducing the amount of food provided socially housed primates to reduce waste will in no way modify what a dominant individual receives or wastes (food is still superabundant to the dominant) but may place a subordinate on a deprivation regimen. Additionally, putting all of a resource in a single location sets the stage for competitive conflicts and dominance turn taking whereas widely scattered resources tilt the balance toward scramble competition as individuals individually forage in separate locations.

1. Aggression between Pairs of Animals

Since dominance is a means of conflict resolution there should be fewer severe fights among well-established groups and severe fights when individuals without established dominance relationships are placed together. Some of this fighting will be a direct result of competition in the absence of dominance relationships and some of it will be due to dominance contests as individuals assess the consequences of fights with one another. Note that it is the monkey that must do the assessment, the monkeys must establish the relationship for themselves. Sometimes this can be done with a single glance as a socially experienced smaller individual immediately yields to a much larger individual that may, in turn, be satisfied with the instant recognition of status. Sometimes individuals may contest their relationship long after it should have been clear to them. This is often the case with socially inexperienced monkeys who do not seem to be able to recognize their own defeat and will persist in seemingly suicidal attacks against a physically superior rival. They also may not recognize submissive signals from a defeated opponent and persist in aggressive assertion long after a normal monkey would have accepted the subordinate signals of a defeated rival.

It is when two animals are first housed together that the worst fighting should occur. In some species, however, where adult members of the same sex may be incompatible (e.g., hylobates, some callitrichids, some cebids, and some guenons), aggression will not be controlled by a dominance relationship and fighting may slowly escalate as one individual gains the upper hand and continues to drive the other away in a situation where the defeated individual cannot leave. In species with dominance relationships, however, we should expect aggression to decline once such relationships are established.

Reinhardt *et al.* (1988) pointed out that dominance relationships can often be established between individuals without contact aggression. If two individuals are placed in visual contact with one another, sometimes one assesses the other based on size, behavior, and other cues as a superior. In such cases a clear asymmetry of agonistic exchanges exists before actual physical introduction. Whereas such assessment is sometimes nearly instantaneous and could occur at the moment of an introduction, some require a little time and can more safely be permitted at a distance. The absence of an asymmetry in agonistic exchanges almost guarantees that some mutual aggressive behavior will occur when a pair is placed together for the first time. A brief contest may quickly establish dominance or the pair may fight on longer if they are more evenly matched. Once the fight has been joined the victor may be expected to engage in more aggressive behavior to reinforce the relationship even after the defeated opponent has reverted to clear submission. If the physical facilities do not allow for either flight or space to indicate submission and chases to show dominance, contact forms of aggression are to be expected. A preintroductory period of visual assessment may be very useful in assessing the likelihood of aggressive conflict on actual physical introductions, but it in no way guarantees that the individuals will fight less than if they had never seen each other at all. I. S. Bernstein (personal communication) has witnessed two monkeys housed in adjacent cages develop greater and greater antipathy toward one another as each threatened the other, lunged at, and attempted to grab at the other or succeeded in biting the other's fingertips. After several days of hostility with no clear resolution one can be

guaranteed of a far more serious fight than if neither had had the experience of displaying aggression toward the other with impunity. Each is "dominant" and each is attempting to maintain "dominance" over a rebellious "subordinate."

2. Group Formations

When more than two individuals are to be housed together, a period of prior visual familiarity, or even prior paired caging, will not establish dominance relationships or preclude initial fighting. Primates are social animals and are skilled in the use of social techniques to attain their ends. A period of paired caging may establish a series of paired relationships whereas in a more complex social group individuals will form coalitions and alliances, usually with a former partner regardless of which was dominant, and will jointly attack rivals to establish a new set of dominance relationship, based on the power of social alliances. Shifting alliances will prolong the period instability.

In forming groups of three or more then it may prove best to do that which intuitively seems worst, to introduce a group of unfamiliar individuals to one another simultaneously. Each individual must assess all others simultaneously. No prior relationships can be used to facilitate joint attacks on another; no individually sustained attack on a single victim is possible with $N-1$ others also needing to be dealt with and no cohesive group attack on a single victim is possible since there are no cohesive alliances to organize aggression. What follows is absolute chaos. Many brief fights break out, often simultaneously. Individuals may form temporary alliances to assert themselves over a victim but the allies must be wary of each other until they have an established set of relationships. The sound and sight of so many simultaneous fights may lead one to believe that this uncontrolled and disorganized fighting will lead to far greater injury than if the introductions were more managed and a staged series of controlled introductions was substituted.

Introduction of animals to one another one at a time over successive days leads to much more organized aggression. As each individual is introduced into the group it will be attacked by those already resident in the group. The residents with newly established relationships will reinforce their alliances by jointly attacking and defeating each newcomer. Each singly introduced animal is a single target to focus their aggression on and will receive concentrated and sustained attacks undisturbed by the distraction of other potential victims. The dominance relationships established may be highly predictable with each newcomer being defeated by the residents, but the last individual to be introduced will have $N-1$ individuals focusing their aggression on them and there will have been $N-1$ fights over as many introductions. The fighting will have been organized, allowing one to monitor closely the course of each fight, but the cumulative injuries probably will exceed that produced when N individuals are simultaneously introduced to one another and only a single period of disorganized and diffuse fighting ensued. In both cases close monitoring of the group is necessary and a decision will have to be made if a particular individual is receiving sustained injurious attacks, such that it needs to be removed for its own safety. Such individuals cannot be returned after treatment for they will not have been integrated into the group and will be introduced into an established group as the last introducee and will suffer renewed joint attack.

Variations on the one at a time and all at once approaches are possible. Introductions can be built up, as small subsets of animals are introduced to one another, then the subsets are combined. Here two alliances are pitted against each other. Since each alliance has multiple targets to attack instead of a single individual to concentrate on, this may prove superior to the introduction of a single newcomer. However, each subset is an organized group that will act jointly in directing aggression toward victims, thus concentrating aggressive attacks on victims even if moving first to one and then to another.

Introducing several unfamiliar animals simultaneously to a group ensures that only the residents will display organized aggression. They may be predicted to win over the newcomers. Introducing several animals to an established group is probably better than introducing them one at a time where they will each be the focus of attack, but organized aggression always has a greater potential for wounding than disorganized aggression.

Most of what has been discussed here has been in reference to extensive experiences with macaques. Naturally there will be species, age, and sex differences. The time course of initial fights with macaques usually includes about 20 min of intensive fighting and then interrupted periods of aggression for the next hour. After this, the frequency of serious fighting slowly declines, but significant fighting can still be expected for at least the first few weeks following an introduction. After that agonistic frequencies are not greater than in established groups. In some species such as *Macaca arctoides*, initial interactions may include very high frequencies of pseudosexual behavior whereas in others, like *Macaca nigra* or *Papio mandrillus*, a long period of tense assessment may precede the first fight. Williams and Abee (1988) showed that aggression levels were still elevated 6 hr after group formations on *Saimiri*. As mentioned previously, an apparently uneventful introduction of two adult male gibbons may be followed by a slow escalation of aggression interactions over a period of weeks and some "compatible" pairs of males, or females, may prove incompatible when a member of the opposite sex is introduced.

Age and sex both have a strong influence on the course of introductions. In many species immature animals with normal social histories have an almost unlimited tolerance for each other. In addition, immatures are often readily accepted into established groups. The physical potential for serious injury exists when small immatures are introduced to adult animals but the very magnitude of the inequality seems to preclude a hostile reception. Xenophobic responses are usually more intense toward adult members of the species of the same age–sex class as the residents. *Saimiri* and *Miopithecus* are obvious exceptions where adult females display very little tolerance to-

ward adult males except during the breeding season. Even rhesus monkey females display much greater hostility toward unfamiliar rhesus males in the nonbreeding season, but seldom with serious consequences to the males unless the females succeed in inciting the males to fight among themselves.

In many sexually dimorphic species there is much greater concern for aggressive behavior displayed by adult males. Males are considered to be potential killers of infants and are often considered to be more aggressive than females. In reality, it is the consequences of male aggression that are more serious rather than the frequency or likelihood of male intragroup aggression being greater. Although instances of infanticide have been reported, male paternal behavior toward orphaned infants has also been reported. Male macaques, at least, are more likely to ignore a newly introduced immature monkey or to protect it against the investigator rather than attack it. Male aggression, directed against other males usually, can produce serious and sometimes lethal wounds in a very short period. Females, without the capacity to inflict the same kind of damage in such a brief period, are therefore often not given the attention they deserve. In many matrifocal social groups, females are far more intolerant of unfamiliar females than are males toward unfamiliar males. Whereas female aggression cannot produce the same severe wounds with the rapidity of males, female aggression is often more sustained and the persistence of their attacks may more than compensate for their lesser strength and biting potential. Social instability among females may be far more serious than instability among males and deserves prompt attention. Whereas some squabbling is typical as young females claim their adult dominance ranks in a group, any instability among the fully adult members of different matrilinies may produce extensive widespread injuries. Females, organized into matrilinies, display organized aggression toward other matrilinies during periods of social upheaval, and the consequences are more widespread and serious than those of a fight between two individual males jockeying for position with one another.

The age variable usually focuses our attention on prime adults, but some laboratory experience suggests that young sexually mature males may be more of a management problem. These males are either victimized by the adult members of a group or, in the absence of adult males, engage in much more aggression toward adult females that they are just beginning to surpass in size. Here perhaps it is the near equality in size that exacerbates the situation as young males lacking full confidence in their ability to dominate females vigorously assert themselves over females that may enlist other females to suppress the attempts of the male. Fully mature males seem to have fewer conflicts with adult females.

Finally, before leaving the age variable, it may be useful to comment on the somewhat more mellow disposition of aged macaques. Senior adults are often slower to incite to aggression and may be remarkably tolerant of immature individuals and one another. Since adult animals sometimes interfere in the conflicts of younger animals, adult animals, and perhaps especially senior adults, may reduce the aggression that occurs among juveniles and adolescents. Again, the inequality in size between juveniles and adults is not an indication of severe unequal aggression but acts to limit the escalation of aggression since the inequality is readily perceived and accepted by socially experienced animals.

3. Intragroup Aggression

Once social groups are established, one will occasionally wish to remove an individual for routine examination, for experimental measurement, or for treatment of illness or injury. Primates can be readily trained for routine capture and examination. They also have sufficient long-term memory so that they can be returned to a stable group after an absence of some time and resume their social position.

If an individual is removed and the group reorganizes in their absence the individual cannot simply reclaim its old social position. It will be rebuffed when it tries to reclaim a social position that now no longer exists in the new social structure and must be reintegrated into the group. Even though it still has some former allies in the group, this is not identical to a new introduction, but it is not as simple as returning an animal to a stable group.

Similarly, if the social position of an animal becomes unstable and fighting due to this instability results in a wound that the animal must be treated for, expect a renewal of fighting on return if the individual was removed before the conflict was resolved. In such circumstances a judgement may be required whether the individual can wait for treatment until the situation has stabilized or perhaps receive treatment and be returned within an hour or two. Repeated captures may mean more work in terms of follow-up treatment. It is more convenient to monitor the recovery of an animal in an isolated small cage, but the dully recovered animal may suffer additional wounds on return or may have to be permanently removed due to the vigor with which its return is resisted.

In breeding groups, individuals are added to the group by births and groups may grow accordingly. Immature animals growing up in such groups have the full range of normal social experience and generally make excellent breeders themselves. With the support of mothers, sisters, and grandmothers even primiparous females generally do well whereas without female kinship units primiparous animals often do less well than multiparous females. The removal of individuals from a group seldom disrupts the social structure. Such removals are artificial "deaths" and the social organization of primates can withstand such losses without major disruption. One might think then that, once established, breeding groups should only be manipulated for harvesting. Some groups, however, may require harvesting not merely to provide the experimental animals desired but also to control group size and to maintain a demographic composition that promotes social stability. Excessive numbers of one

sex or excessive numbers in a particular age group (e.g., adolescence or old age) may signal the need for adjustments.

In addition, one must always be concerned for the long-term consequences of breeding group management. Whether inbreeding leads to any undesirable anomalies or breeding depression, one may wish to maintain genetic heterozygosity and to maintain a representative population instead of developing an inbred strain. Such considerations must consider the average breeding age of the more rapidly maturing sex (generally female) and that there are no demonstrated behavioral mechanisms that preclude inbreeding in primates living in captive groups. The normal mechanism of genetic exchange involves the transfer of members of one (sometimes both) sex between groups at, or shortly after, puberty, and some later less predictable exchanges of adults as well. Since such exchanges by the animals are precluded by caging, one must prepare a procedure for periodic genetic exchange. First, there will usually be fewer breeder males than females in the group and the social relationships among males are less crucial to group stability (true for most macaques at least). It is therefore easier to exchange breeder males at some predetermined intervals and to harvest out maturing males. In introducing the new breeder males, remember that xenophobia is sharpest toward same age–sex class individuals, suggesting that all resident breeding age males should be removed prior to the introduction of the new breeder males. Second, females are often more tolerant of new males during the breeding season and so new males may be more acceptable at this time. Third, the residents usually support one another against intruders and so a period between the removal of the former resident males and the introduction of new males may be useful in preventing female aggressive responses against new males in support of resident males who were removed only a few minutes or hours ago.

All of the just-mentioned discussions are more or less in the way of accumulated laboratory wisdom. Some of it follows directly from empirical studies but we can never be certain that we have a final answer to the problem of controlling aggression. Future experience will only refine our knowledge and techniques. We may constantly discover exceptions and special considerations that apply to a particular species or animals living in a particular setting. Hasty generalizations both in adopting new procedures because they worked once somewhere, and in scrapping old procedures because they sometimes fail should be avoided. The solutions are far from perfect but one should try to develop the best available. Our procedures should be selected because they generally result in the best managed and maintained animals. Short-term solutions often result in long-term problems, as is true of harvesting animals early to reduce maternal interbirth intervals; a procedure that succeeds at the cost of the next generation of breeders. Solutions to the second problem (peer rearing, surrogates) are not nearly as successful as normal social rearing and we increase our labor input with no gain, sometimes with a loss in productivity. In housing animals that may live as long as 30 years (monkeys or apes), one must take into account the long-term perspective.

B. Maternal Care

Perhaps the most important bond between any two mammals is that between a mother and her infant. Primate infants are born helpless and would soon die without maternal care. The mother is the primary socializing agent in the life of a newborn. Socialization is a lifelong process by which individuals learn social skills interacting with members of their species. This learning takes place through observation of others and through direct participation in daily events. McKenna (1982) lists five major areas that mothers contribute to the care and socialization of the infant: nutrition, transportation, protection, social support, and emotional support.

In the captive environment it is often necessary to intervene in the normal mother–infant relationship. Whether for reasons of maternal rejection or experimental necessity, nursery rearing is the only option for some infants. In most cases the long range goal is to return the animals to the laboratory as "normal" subjects or for breeding purposes. Abnormal rearing conditions lead to abnormal behavior as an adult. Only by providing for each of the infants needs can a nursery-rearing situation successfully complete this goal.

The primary contribution of the mother is nursing and support. Before weaning, the mother is often the sole source of nutrients for the infant. During and after weaning the mother acts as a guide in providing information to the infant on what to eat. The infant also may benefit from receiving hard to get foods from the mother. In species where direct competition for food is strong, the mother may prevent the infant from being chased away from food items.

Transportation is another way the infant is assisted by its mother. Most primate females carry their infants either dorsally or ventrally. Some species of the nocturnal foraging Lorisidae will park their infants in a safe place while they go out and forage. As the infants develop and become more secure, the amount of time spent on the mother will decrease. This continues until, after weaning, most of the infant's time is spent in proximity to the mother, not clinging. Hinde and Spenser-Booth (1967) provide a good way to measure the changing relationship between mother and infant. By comparing the number of times the mother approaches and gathers in her infant to the number of times the infant initiates the contact, ratio values can be developed indicating the locus of control for the interaction. Values less than one indicate maternal control and values greater than one suggest infant control. As the infant becomes more secure in the environment the ratio should shift from maternal control to infant control. Comparing this to a similar ratio of rejections of control, times the infant or mother wants to cling but is refused, provides a picture of the conflict that can

occur during the weaning process. During weaning the rejection ratio increases as the mother more often refuses to carry or nurse the infant. Simultaneously, the number of clinging attempts by the infant should increase. The exact pattern seen probably will be fairly uniform across most primates with major changes only in the timing of events.

Infant primates must learn what is dangerous and what is not. This is true of social stimuli as well as environmental stimuli as the mother provided a readily available source of information. By removing the infant from situations and punishing the infant for certain behaviors, a mother modifies the actions of her infant. The mother also can modify the actions of other group members toward her infant. In most primate societies that develop around matriarchal groupings, the dominance ranks of the infants are directly related to their mothers. This type of dependent ranking was first demonstrated in Japanese macaques (Kawai, 1965; Kawamura, 1958). It has been suggested that the infants learn who is dominant to them and whom they can dominate by how the mother aids them in agonistic encounters; either she attacks their opponent or carries them away emitting submissive signals. Within a matriarchy the younger females will generally rank over their older siblings because the mother will aid the youngest animal against the older. Thus both the offspring learn that the younger is dominant.

The concept of emotional support deals with the attachment that develops between the mother and infant. Attachment was first discussed by Bowlby (1969) and Ainsworth (1972) as an affectional bond between the infant and mother. If an attachment has formed between two animals they will be able to differentiate each other from other animals. Each will act as a source of comfort to the other. These notions were explored by Harlow (1969) before the concept of attachment had been formalized. In his classical set of studies he demonstrated that this "contact comfort" was more important to the infant than the availability of food. When given the choice between two surrogates, one made of wire that provided a nipple for sucking and the other covered in a soft terry cloth, the infant consistently chose to cling to the soft-covered surrogate over the one that provided the milk. If the soft surrogate was removed the infants would go through a syndrome of distress and agitation that finally led to despair and lethargy. Further studies found that the closer the surrogate was brought to acting like a real-life mother, combining soft fur with milk and movement, the stronger was the attachment developed by the infant.

These factors become very important when designing a primate nursery. For a nursery-reared animal to be a functional member of a primate group it needs to be reared in as close to a natural environment as possible. Infants reared in total isolation, without the contact comfort provided by a proper surrogate, exhibit a wide range of abnormal behaviors, from self-clasping to eye poking to stereotypical rhythmic movements. When these animals are placed back with others they do not show the normal interactive patterns; juveniles will launch suicide attacks on larger adult males and refuse to stop attacking. Providing the infants with socialization will help integrate them into a social group. Depending on the experimental needs, socializing the infants to human contact may be the most appropriate course. Constant human contact should make the animals more trusting and adaptable to experimental situations that involve human interactions, such as certain learning tasks or testing apparatus.

C. Alloparental Behavior

Alloparental behavior can have a profound influence on the socialization of an infant. Alloparental behavior, or "aunting," is usually defined as behavioral patterns typically seen between mother and infant shown between any other animals and the infant. Whether alloparental behavior occurs or not can alter the types of social interactions an infant encounters. It also can provide additional secure bases from which to explore the environment. The extent to which alloparental behavior is seen varies greatly from species to species. Langur and squirrel monkey infants spend as much as 50 and 30%, respectively, of their time on alloparents whereas chimpanzees only spend 1% or less. In those species that display alloparental behavior it is typically not random. Factors such as the age of the infants, maternal ranking, kinship, and the age and experience of potential alloparents all play a part in determining if the response will occur. Species where females stay together in extended matriarchies might be expected to show more alloparental behavior than where females spend much of their time alone, as in chimpanzees. The social structure of the species also plays an important part. Among the marmosets and tamarins the father spends a great deal of the time transporting and caring for the infants. Epple (1975) has shown that early experience is necessary for the males to exhibit these responses. Why certain species display a great deal of alloparental behavior and why others in a similar ecology and social structure display very little is not clearly understood. Several theories have been proposed as to why alloparental behavior should exist at all. The experience might act as practice for young nulliparous females. Infants may enhance the status of the alloparent by allowing her to elicit aid for the infant's mother or by acting as a buffer between the alloparent and an antagonistic individual. This type of "agonistic buffering" has been suggested as one function of barbary macaque males carrying infants.

A number of individuals could benefit from alloparenting. Mothers show increased foraging efficiency while their infants are away. The infant may benefit by exposure to more or different social situations that may hasten socialization. Related animals should benefit as the behavior increases the infant's chances of survival and reproduction. It has been hypothesized that alloparental behavior could increase an infant's chances of adoption if the mother died. This has been seen only rarely. Alloparental behavior could put the infant at a severe disadvantage if the alloparent is an inexperienced young animal.

McKenna (1982) has also suggested that alloparental behavior could aid in the establishment and maintenance of female–female bonds. These bonds are important in many socially living primates and act as the central glue to the solidarity and cohesion within a social group.

VIII. CONCLUSIONS

The purpose of the preceding chapter has been to provide a background in animal behavior, in particular how one should address behavioral aspects of primates in captivity. The single most important adaptation of the primates is their sociality. Although they are not the only mammalian order to develop highly involved social mechanisms, they are perhaps the most dependent on joint action as their primary adaptation. Specific expressions of sociality vary and the variety of social structures and organizations within the order Primates make general statements very difficult. Even within a genus, species have adopted very different social styles that make it dangerous to say one knows how "baboons" or "galagos" behave. The differences between the social organizations of species within the same genus, such as hymadryas baboons and olive baboons, may be just as great as the differences seen between macaques and capuchin monkeys, members of different suborders of primates. The need to "know thy animals" is paramount to proper handling in any social species, and is particularly true for primates. One should always refer to the primary literature as a basis of information when using a new species of primate. Lessons learned from experiences with one species rarely can be generalized for another.

The use of behavior is important to the general management of primates in a captive environment. Daily observations of the animals by specific people are important. The animals become individuals and it is possible to develop profiles of animals that will make clinical and experimental decisions easier to make. Timid or asocial animals may not be appropriate for introductions to new social groups, whether it is for breeding or experimental use. These techniques will prove of value to the husbandry personnel and may be of importance when choosing animals for experimental use.

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CHAPTER 6

Environmental Enrichment and Psychological Well-Being of Nonhuman Primates

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I. INTRODUCTION

Experience suggests that most specific rules for the housing, maintenance, and treatment of nonhuman primates in the laboratory have been, are, and are likely to continue to be in a state of flux. Regardless of the particular components of the regulations which emerge at any given juncture, common goals are evident in the regulations and the activities of the scientists and animal care professionals who work with primates. These common goals are to structure the settings within which primates are kept to achieve optimal physical health, while sufficiently engaging the full capacities of the subjects so as to sustain similarly high levels of psychological well-being. The specific means to achieve these goals do not yet reflect a scientific consensus (Dahl, 1982; Schmidt and Markowitz, 1977; Maple and Finlay, 1986; Holden, 1987, 1988; Moberg, 1987; Irving, 1985); furthermore, the issues involved are not definitively legislative. Regardless of the turns which legislation may take in the coming years, if these ultimate goals are to be met, a number of basic perspectives must be maintained; a series of conceptual themes which have emerged in recent research also must be incorporated into the efforts. Instead of attempting an encyclopedic review of a rather disparate literature (Segal, 1989; Fajzi *et al.*, 1989; Bayne, 1989), this chapter attempts to highlight the conceptual issues which are central to the objectives in enriching the lives of primate subjects.

NONHUMAN PRIMATES IN BIOMEDICAL RESEARCH: BIOLOGY AND MANAGEMENT

II. DEFINITION OF PROBLEM AREAS

A. Commitment to Animal Welfare within Scientific Community

To continue the complex task of determining how we may best enhance the psychological well-being of captive nonhuman primates most effectively, we must reject the polarizing idea that there are two opposing groups in contention for this determination: the "animal welfare" group and "animal scientists." Those who spend their lives working with, studying, and curing animals must ultimately serve as a primary source of the information essential to any effort to understand those features of the environment most conducive to primate welfare. Negative accusations regarding animal scientists abound in the public media. Nonetheless, we must approach this problem armed with the conviction that committed concern for the well-being of primate subjects is overwhelmingly true throughout the community of veterinarians, scholars, and scientists working with these, as well as other, species. The entire animal research and animal care community has been responsively sensitized by the thoughtful challenges of individuals, both within and outside the scientific community, who have stressed the need for continuous vigilance in attending to potential improvements in the conditions under which animal subjects live and the ways in which they are treated. The majority of scientists studying nonhuman primates and of biomedical professionals concerned with the psychological and physical health of primates have traditionally been concerned with the "welfare" of their subjects. Because these professionals hold these commitments—blending an emotional concern for the animals with an appreciation of the incalculable advances in human welfare which depend on the valid study of them—the primate research and veterinary community has proven itself eager for the basic information relevant to its continued goal of achieving the best possible conditions for the maintenance of captive nonhuman primates. In this sense, we are all members of the "animal welfare" community.

The differences in perspective which emerge among the groups concerned with the welfare of nonhuman primates generally spring from two sources: (1) the basis (i.e., either objective or subjective) on which the poorly understood term, "psychological well-being," is to be judged; and (2) the priority attributed to particular animal care/treatment considerations in comparison to the significance seen in the benefits for humankind from research requiring delimited compromises in ideal conditions. Although a discussion of the relative value of human and nonhuman life is ultimately central to the second issue (McCabe, 1990; Sackett, 1988; Fox, 1983; March, 1984; Bowd, 1980; Dragstedt, 1960; Beauchamp, 1985), it clearly goes beyond the scope of this chapter. Our discussion, therefore, will be directed toward the first of these differences in perspective. The creation of environments serving both essential human informational requirements and the psychological needs of subjects from whom this information is sought requires an empirically based, rational perspective.

B. Mandate for Environmental Enrichment

As a society, and as individuals, the need to establish the most appropriately humane means of housing and utilizing nonhuman primates for research and educational purposes derives from both rational and emotional perspectives. On the one hand we wish to do what is "right" for the animal to reduce any unwarranted stress or harm, while on the other hand we recognize that the validity, and hence the value, of the work with these animals depends on the physical and psychological status of the subjects. In examining the ways in which our relatively more subjective judgments are made, we do not denigrate emotional responses or the people who are subject to them; rather, we seek to determine whether these all-too-human approaches are actually likely to achieve the goal, i.e., the psychological enhancement of the well-being of captive nonhuman primates.

The philosophical debate over the possibilities of "knowing what another feels" is clearly relevant to our considerations here (Dawkins, 1990), but go beyond the scope of this chapter. Instead, we will consider several of its most salient features in relation to the practical difficulties which confront those seeking to enhance the lives of primate subjects. In general, we seek to understand the reactions of other individuals of our own species by referring to our own internal responses to particular situations and events. We assume a degree of parallel response in others because they are, or we believe they are, pretty much like ourselves. We tend to feel more confident in making such judgments when others are most like ourselves (e.g., those of the same sex, age, race, or culture). Notwithstanding the problems we might have at times, we work on the assumption that most "normal" people are pretty much alike. To be sure, it is commonly recognized that very young children and adult sociopaths may have little ability to appreciate and be affected by the feelings of others. But as a society, we expect and value the emergence, initially, of the ability to sympathize with others in difficulty and, finally, of the ultimate achievement of human interaction, the ability to empathize with others—actually feeling what it is like to "be in another's shoes."

Unfortunately, experience makes it clear that these social processes are all too subject to error. Men and women may each have difficulty understanding the feelings, needs, and preferences of the members of the opposite sex of their own culture. Parents, but a single generation removed from their progeny, often fail to understand what motivates and satisfies their own offspring, whereas the latter seem at times to regard their elders as if they were of a different species.

At larger societal levels, the history of our country, as one example, shows that as each new immigrant group arrived, their predecessors found the values, interests, and behaviors of the

newcomers alien in far more than a mere geographical sense. Not just language, and hence direct communication, is impaired between these groups. Often a lack of understanding of the fundamental features of the environment which move/deter and frighten/delight these distant individuals is central to the difficulties of even the best intentioned of both sides, to truly empathize with the other. There are many other current examples of these problems in understanding the needs and perspectives of "other" groups. Large housing projects designed to provide better housing for the poor are being torn down because they were unworkable and unreliable; mental patients are not faring better following "decentralization" which removed them from large institutions; and people on inadequate diets neglect nutritious food which fails tofit their cultural preferences. Good intentions and our best intelligence are clearly not enough. If problems are prevalent when we attempt to determine the salient features of the lives of other members of our own society, then surely we should be extremely cautious in approaching any judgment of the presumptive needs and preferences, not merely of a single form rather different from ourselves, but the unquestionably wide-ranging characteristics of all the members of a whole order of animals.

Not only do human groups differ in needs and perspectives, but they may also differ in perceptions as well. For example, the eskimo may detect and be affected by variants in snow patterns that escape the average urban dweller. Similarly, the urbanite may detect and respond fearfully to an individual suspected of posing a danger, whereas the rural visitor may see nothing worthy of avoidance in the same stranger. These often profound differences in perception are based on experiential factors alone. Should it be any surprise, then, if the various primates we study when placed into diverse laboratory settings may quite literally see, hear, and otherwise respond to stimuli which carry different meaning to the human observer trying to empathize with them?

The problem of anthropomorphism is exacerbated both by the fact that the cognitive and affective capacities of these nonhuman primates are likely to differ considerably from our own and by the fact that the sensory world of these subjects can differ from that of humans in a number of salient dimensions. High frequency auditory capabilities, olfactory capacities, and the general likelihood that various perceived features of the environment do not simply parallel those of the average human observer complicate the task of grasping the perceived world of these animals.

As von Uexkull pointed out many years ago in his descriptions of differences in "umwelt" (the perceived environment) among various species, we can expect that armchair, anthropomorphic assumptions about the experiential world of our nonhuman primate subjects are fraught with error. As von Uexkull described so vividly:

> We are easily deluded into assuming that the relationship between a foreign subject and the objects of his world exists on the same spatial and temporal plane as our own relations with the objects in our human world. This fallacy is fed by a belief in the existence of a single world into which all living creatures are pigeonholed. This gives rise to the widespread conviction that there is only one space and one time for all living things.

Von Uexkull goes on to say, "The first task of Umwelt research is to identify each animal's perceptual cues among all the stimuli in its environment and to build up the animal's world within them. . . ." (von Uexkull, 1934, in Schiller, 1957)

As this early student of animals in relation to their environment made clear, it will only be through careful and rather creative experimental studies of the effects of diverse stimuli, organized in a variety of ways, that we will be able to resolve these interspecific differences in psychological perspective and responsivity.

1. Criterion of "Naturalness"

It is clear that we cannot generally make simple anthropomorphic projections of our own reactions to given situations as the likely equivalent to those of our nonhuman primate subjects. Unfortunately at present we also lack the critical empirical data which might allow for rigorous and systematic appreciation of the diverse perceptions and psychological needs of these animals. Nonetheless, we often find those interested in these problems making personal, "esthetic" judgments about what should be best for these subjects.

It appears that the most frequently sought basis of this esthetic judgment is the animal "in the wild"—"nature" being the ultimate source of what is good, bad, or indifferent in its positive or negative impact on the species in question (Maclean *et al.*, 1987; Hancocks, 1980; Beaver, 1989). In its simplest form, this approach suggests, for example, that a tree limb should certainly be a better perch or climbing device for the average primate than an aluminum bar or PVC pipe, even though the animal may actually prefer the pipe (Williams *et al.*, 1988). But, unfortunately, this zeal for encouraging the replication of the features of "the wild" in our laboratory settings, as a primary means of enhancement, generally fails on two significant counts. First, while many of us may be seduced by the superficial appeal of these naturalistic assumptions, they are, in most instances, just exactly that: assumptions as to what is *natural.* As a result of human incursions, the settings in which contemporary primates are found are often drastically different from the environments within which the evolution of these animals took place. Furthermore, because of the tremendous diversity of environments for *different species*, as well as the environmentally diverse dispersal *within each species*, we often have little to guide us in making decisions regarding the "natural environments" we seek to emulate in the laboratory.

The second point on which the criterion of naturalness fails is that regardless of the aspect of the nominally natural environment we examine, we know that many of these features are not ubiquitously benign (Welshman, 1985; Kessler *et al.*, 1986). For example, virtually all the features on which life depends,

including food, water, nesting sites, and suitable mates, may place tremendous stress on wild animals when they fall into scarce supply. In addition, those espousing the presumptive virtues of a naturalistic setting certainly would hesitate to suggest the imposition of these adverse environmental events into laboratory settings. Similarly, while most free-ranging primates (but by no means all) live in social groups, ranging from a few to over a hundred, not all members of these groups participate to the same degree in the presumptively salutary effects of these societal organizations.

Those at the bottom levels of the social hierarchy are generally displaced from desirable feeding or resting sites, are more often injured, may have relatively high rates of fetal wastage and neonatal death, and their surviving offspring often leave the group at puberty. Subjects in the somewhat unstable middle levels of a dominance hierarchy, or those otherwise contending for status with others, may, under some circumstances, be under the greatest level of stress. Similarly, those who belong to relatively dominant kinship groups within the troop may have an entirely different benefit of group membership than those of more subordinate lineages. More dominant animals may feed from more accessible sites, obtain richer food sources, claim more secure resting areas, and are generally more capable of otherwise enhancing the cost-benefit ratios of their functioning within a given environment. Moreover, those who, because of a significant injury or disease, are unable to keep up with group movements as a result of the debilitation are unlikely to benefit in the same ways from group membership and the regular social experience sought in many esthetically driven enrichment approaches. Indeed it may well be true that under "natural" conditions, in which human vigilance and intervention is not maintained, many animals, rather than benefiting, actually suffer considerably from "regular opportunities for social interaction" (e.g., Fedigan, 1983; French, 1981; Estep *et al.*, 1986; Southwick, 1967; Janson, 1985).

2. Criteria for Assessing Well-Being

We can attempt the assessment of psychological well-being from one of two interrelated perspectives. One approach would attempt to determine psychological status indirectly, through the assessment of physical parameters which are presumed to reflect psychological functioning. Such an approach has the immediate advantage of providing relatively objective criteria for evaluation on the basis of already known factors in the etiology of many disease states. The alternative or complementary approach views the direct observation of behavioral patterns, both "normal" and "pathognomic," as the primary basis on which well-being should be judged. Obviously, some integration of these two approaches should prove to be the most valuable.

But, consider the perplexing problems which ensue even from the first approach. From a rather anthropocentric perspective, a number of physical features would appear to be clear reflections of well-being, psychological or otherwise. Rates of conception, fetal and neonatal survivorship, rates of growth, onset of sexual maturation, total reproductive levels, longevity, freedom from disease and injury, as well as health of coat, teeth, and other organs, all can be *generally* enhanced by captive conditions when those conditions are created on the basis of a substantial body of empirical data developed over a number of years. Certainly an animal may suffer drastic reduction in any one or more of these physical parameters when intuitive judgments are used in place of reliable information or when such data do not exist.

Nonetheless, it may be claimed that a physically sound, disease-free primate is not necessarily a psychologically healthy primate (e.g., Tigges *et al.*, 1988). From this perspective, however, the physical well-being of these subjects still should be taken as fundamental. Once physical health is achieved, further manipulations of the captive environment and treatment of these animals should be focused on some as yet undefined improvement in psychological functioning. But this approach still leaves us the problem of determining what dependent measures can be considered as good candidates for our assessment of psychological well-being. Thus, this line of inquiry is fraught with all the problems inherent in attempting to make broad cross-species assumptions and then acting upon them. In light of our current and clearly limited state of knowledge, the current authors, recognizing the difficulties in attempting to transcend these inherent difficulties, strongly endorse an exploratory, research-based approach to all aspects of the functioning of different species in various group and single cage settings; this approach should be cautious, but wide-ranging. It seems likely that in the absence of a sound research base, polemics rather than science may set the paths we follow in the future.

One potentially useful technique emulates that of the modern diagnosis and treatment of human psychological disorders. Following this system, in our effort to determine conditions to be avoided, as well as those to be encouraged, we seek, on the one hand, to define a set of both positive and negative "symptoms" of primate psychopathology. On the other hand, we seek at the same time to identify a series of functional parameters which are likely concomitants of psychological well-being.

A set of behaviors exists for which there is good agreement that they represent the positive symptoms of chronic behavioral pathology in many primates (Paulk *et al.*, 1977; Preilowski *et al.*, 1988; Bryant *et al.*, 1988; Goosen, 1988). These include behavioral stereotypes such as repetitive rocking in place, chronic self-clasping, -pulling, or -slapping, so-called "floating limbs" (in which a limb may seem to move "of its own accord" and be stared at or even grabbed by the subject), and the so-called "cage stereotypies," in which an animal repeatedly tracks the same path around some portion of its enclosure. Even more extreme examples of behavioral stereotypies which may be seen as reflecting pathology include self-biting, prolonged autoerotic activities, self-orality, and hair-pulling.

Negative symptoms are somewhat more difficult to define since they represent a cataloguing of those patterns in which an animal should engage or be able to engage, but which it fails to

perform. A member of a generally gregarious primate species who cannot live with another animal because of excessive fear or aggression, a male unable to copulate or simply disinterested in receptive females, a mother unable to nurse or nurture her infants, an animal continuously avoidant or quite frightened of simple inanimate objects readily approached by other members of the species, or the subject unable to learn relatively simple tasks or reward sequences—each of these is a negative symptom which strongly suggests *chronic* pathology. But unfortunately the determination of other negative "failure-to-respond" symptoms, which might even more sensitively detect chronic psychological disability, still awaits examination of a wider range of reliably assessed psychological capacities. As in assessments of human psychopathology, proving the existence and significance of negative symptoms is always difficult.

But what about more *acute* psychological symptoms of psychological distress? This can in fact be an even more complex problem. Certainly the animal sitting motionless in the back of its cage, hanging continuously from a precarious position, or suddenly unwilling to eat or drink normally may be experiencing some degree of acute psychological disturbance. But ruling out physical illnesses which may produce the same symptoms must be done before a diagnosis of psychological disturbance can be made. Although certain acute physiological measures, most particularly cortisol measurements (Gonzalez *et al.*, 1982; Hennessy and Levine, 1979) and more recently some immunological parameters (Coe *et al.*, 1988; Kelley, 1985; Coe *et al.*, 1985), appear to be reliable reflections of short-term psychological stress, other indications are more difficult to interpret. Repeated vocalizations may be a good positive symptom when they appear, but the absence of vocalization in the same species does not certify a lack of stress (Levine *et al.*, 1984, 1985). Although eating and drinking may be disrupted under some demanding conditions, under other conditions ingestive patterns may be poor reflections of psychological states. For example, when separated from their mothers for several days, most infants, despite other indicators of rather profound psychological disturbance, will eat and drink eagerly (Rosenblum and Kaufman, 1968).

C. Factors Influencing Establishment of "Standards"

1. Consideration of Species Differences

Regardless of the sources of information which will serve as the basis for establishing suitable environments for captive primates, as part of a rational approach we must formally recognize the very considerable differences which exist among different species of primates and often among classes and individuals within each species. Literally hundreds of studies have demonstrated differences not only in size and appearance, but in sensory, perceptual, cognitive, and affective characteristics as well. As a consequence, imposition of standards that are valid in meeting specified goals for the "generalized primate," on the one hand, may be no better than a "lowest common denominator" approach or, on the other hand, may serve as a straitjacket into which all species are forcibly squeezed. Thus, whereas "floor space" may be of paramount concern to the terrestrial human and other similar primates, available climbing height may be most comforting to an arboreal monkey. Similarly, one species may prefer to live in relative isolation, whereas many others are strongly gregarious. The phenomenon of "individual distance" (i.e., the distance between individuals of a species within which specific behaviors, such as aggression, may be triggered, and other patterns, such as feeding, may be inhibited) clearly differs dramatically among species, even within the same genus. Pigtail macaques, for example, rarely sit in close proximity and virtually never sustain passive contact with nonkin adults, whereas bonnet macaques will spend long periods in close contact, even with relative strangers. Similarly, opportunities for close groupings may be a positive environmental element for females of a species, whereas males of the same species may avoid such close encounters between themselves and avoid close contact with females to an even greater extent; the Peruvian squirrel monkey is an excellent example of this type of sexually segregated social organization. Hence, in this latter species females may receive positive and males negative effects of relatively close contact. Once aware of the species- and class-typical preferences, room design can incorporate these perspectives in various ways. Thus when total floor space is limited and subjects with larger individual distances are housed, psychological and perceptual separations can be employed in lieu of physical space. Opaque, but readily crossable, barriers, pass-through doors and tunnels, and multiple single-subject perches as opposed to larger group shelving can each allow animals in a group to move together in various sized temporary groupings, while allowing individual separations as desired. Less gregarious species, with greater individual distances, may sit comfortably only a few inches apart on either side of a barrier, whereas similar subjects in full view a number of feet apart may remain vigilant and tense. For some species, it would be valuable to assess the usefulness of "smoked" Plexiglas barriers, which can limit access and modulate social stimulation (perhaps emulating ways that foliage can act in the wild), while allowing subjects to monitor salient events within their social environment. Similarly, when possible, multiple feeding sites, at different heights within the pen and on opposite sides of barriers, prevent dominant animal control and reduce feeding stress and aggression in less gregarious species. It is ultimately the perceived, psychological environment which regularly affects behavior.

To be sure, all primates have certain attributes which characterize members of the order Primates. As warm-blooded vertebrates, all primates hold in common a number of basic nutritional, respiratory, and thermal requirements. But even within these objectifiable, quantitatively measurable domains, important species differences exist. Certain lawful relationships, however, hold between different species; this fact can be used to achieve selected goals in maintaining different species under nominally parallel conditions. To take one example, as

with other mammals, the metabolic costs of moving through the environment differ as a function of body mass. According to our best data on the allometry of the nonhuman primates, metabolic rate does not change linearly with size, but rather it changes with 0.75 power of mass. Thus, a species twice the size of another does not incur twice the metabolic "cost" for moving a given distance within its environment. If, as some may advocate, we establish as a goal the provision of each species with a functionally equivalent environment, this metabolic scaling factor must be taken into account. Thus any caging standards, based on some form of linear or simple volumetric approach related to body weight, will hold intrinsic differences in their impact on the different sized species they are intended to accommodate. Reasonable standards must recognize that a species twice the size of another needs more than twice the space of the first if the energy costs in utilizing their environments are to be made equal.

The same approach would similarly affect any decisions regarding the effort we might wish to require of animals in obtaining their daily food, e.g., the distances they must travel or the work they must perform in extracting a given caloric input from their environment. Since costs of performing a given action differ from species of one size to species of another in a systematic fashion, the caloric input resulting from that action should, if equality of treatment across species is a goal, differ in a parallel fashion or else the required work should be proportionately adjusted. Unfortunately, in the absence of this lawful comparative information, generalized regulations stipulating "work" for food or water may in fact unduly stress members of one species while leaving another minimally involved. Certainly, as discussed earlier, there are other objectively assessable outcome measures which could also be used as indirect reflections of comparably desirable environments for different species: general health (e.g., lack of disease or wounding, quality of coat), longevity, reproductive rate, fetal loss, and/or rate of growth, to name several obvious choices. But each of these quite overt measures must also be judged in terms of species-relevant parameters. Given the health problems and reproductive losses that are repeatedly reported in field studies of various species, we may or may not choose to set our laboratory goals and assessments on the basis of our estimates of the characteristics of a given species in nature. There are various ways in which we can choose to incorporate such elements in our determination of the quality of laboratory environments and the life of the primates living within them. But clearly, objective, biologically sound measures afford a more appropriate basis on which such judgments can be made than do the subjective, esthetic perspectives which are often proposed.

2. Use of "Minimal Standards" Approach

This dimension of the problem confronts rather directly the philosophical and practical questions central to the concern for primate well-being. Minimal standards may provide some legal safeguards, but they fall short as a general long-term strategy. Given (a) the ubiquitous constraints on financial resources; (b) the considerable diversity of particular requirements of specific research programs; and (c) the fact that our understanding and definition of "requirements" continue to evolve rather slowly, there are those who feel that as definable standards are developed our first priority should be prevention of the emergence of recognized physical and psychological pathology of the types discussed earlier. Consistent with this position is the view that since we cannot "get into the animal's head," any notion of "optimizing" conditions any further is, in fact, an exercise in the sort of projective analysis which we have already considered. As such, it is judged that the establishment of minimal standards compatible with recognized objective goals should be the goal of any environmental enrichment program. However, it can be argued, in the strongest statement of the opposite case, that given the relative paucity of our knowledge, every effort should be made to maximize the presence of those factors which we suspect may enhance well-being. Thus, more space is better than less, more "positive" stimulation is better than less, and more diversity of stimulation is preferred to less; similarly, other objectives would include less handling instead of more, less stress instead of more, and less population density instead of more. As one might expect, the cautious and more reasonable position may well lie somewhere in between the extreme positions just characterized.

Some features of the environment coerce behavior whereas others make options available to the animals themselves. Thus, other things being equal, an animal is not forced to use more space, nor are groups required to disperse within it. In contrast, when food sources are varied the subject has no alternative but to confront this fact and adjust its behavior accordingly. In general it would appear prudent that we pace our commitments regarding captive environments to those factors upon which solid knowledge is available; we should cautiously extend beyond defined parameters only when efforts to further "optimize" those elements are unlikely to place adverse demands, rather than opportunities, on our subjects. To do otherwise, as with the imposition of any rules derived from beliefs rather than objective data, could well result in outcomes quite the opposite of those intended.

The regulations adopted in 1991 appear to reflect a growing appreciation of an open-ended, more flexible perspective on approaches to enrichment as opposed to the imposition of dogmatic and rigid preconceptions of what should be effective in meeting our goals. The new "performance standards" approach allows for two critically important elements: first it means that environmental features which are compatible with diverse local conditions, particularly research requirements, may vary from laboratory to laboratory so long as criterional standards of subjects' psychological and physical health are achieved. That is, these current regulations place their emphasis and concern on the animals themselves, how well and healthfully they function, instead of on the physical facilities and procedures through which that quality of life is achieved. Second, these regulations allow us to continue to evolve both our criteria for successful

maintenance and the varied means of achieving these goals without the recurrent imposition of newly derived standards devoid of empirical foundation, as has happened in the past. Indeed, we would hope that the inspection and certification system would become a mutually educative process between inspectors and care-giving groups which would allow for the exploration of both the criteria of evaluation and the means of achieving them.

3. Limitations on Employment of "Optimal" Conditions

In keeping with the views just discussed, it must be clear that the revised standards, having been established at the beginning of the last decade of this century, are unlikely to remain intact even until the next century begins. Indeed, we should all hope that sufficient new knowledge will permit a continuous evolution of new concepts and methods designed to enhance the welfare of our subjects. As a consequence, unlike the expectations prevalent only a few decades ago, investment in physical facilities cannot be viewed as a long-term, one-time commitment. An overzealous commitment to unproven housing and stimulation programs may not only bankrupt the biomedical programs they are designed to facilitate, but may in a short time leave an institution further away from its goals than when it began. Thus as manpower costs rise at even faster rates than that of physical facilities, schemes in which animals are individually transported and exercised outside their regular living area on a regular basis must be viewed with caution as well. To be sure, when basic housing conditions do not *permit* "adequate" physical activity, some additional opportunities for activity may be desirable; but, as alluded to earlier, more space does not have to be used by an animal. To coerce physical activity presents enough potential stress problems on its own to preclude this form of response to the problem. Increases in mandated cage sizes, based on human perceptions and projected instead of empirically determined characteristics, may not only waste space and funds needed to substantively enhance well-being, but, if inappropriate to the subjects, may result in unutilized space. Moreover, by expanding the area to be monitored by the animals and increasing the level of vigilance to be maintained regarding the intra- and extra-cage domain, the increases in cage size could actually increase rather than diminish stress. We must first know how animals use available space, what individual and environmental features affect those preferences, and what the benefits and detriments are to the possible alternatives before financially demanding programs of environmental modification should be attempted. This same approach should be maintained regarding all fundamental elements of proposed environmental enrichment programs.

4. Meeting Special Requirements of Specific Research Paradigms

Three of the most frequently suggested dimensions of the environment which have been suggested as likely to provide some significant elements of psychological enhancement include: (a) opportunity for routine social interaction; (b) regular opportunity for physical exertion or exercise; and (c) the employment of manipulanda of various sorts, including foraging tasks. Each of these elements is presumed to actively engage the attention and affective reactivity of the animals and thereby to provide the sort of stimulation necessary to psychological well-being. Although relatively little generalizable information is available regarding the actual impact of these presumed enrichment features on the members of particular species, it is clear that such elements as enforced exercise, massive space increases, or required social housing, at least in the form in which they are often considered at this point, may simply be impossible to incorporate into the environment of the captive primate in all instances.

Most significantly, the opportunity for physical interaction with conspecifics is generally precluded in most forms of research on communicable disease. Although limited pair-housing may be useful in some cases, group housing is certainly antithetical to most infectious disease research protocols. Even periodic contact with a limited number of partners may not be feasible within these research paradigms. Certainly the danger of wounds and related injuries inherent in the social interaction situation would obviate its use in any number of additional research protocols which would be invalidated if such regular events were to occur. Similarly, various types of metabolic investigation, and those studies in which animals are subject to orientative or equilibrium problems, to take two more examples, are not suitable candidates for expanded environmental exercise possibilities either because of the variable rates of energy expenditure or the possibilities of injuries to the subjects.

Furthermore, as alluded to earlier, since animals may choose to use or not use activity opportunities, variability within and across subjects at various points in the experimental protocol may add appreciably to the error variance in such research and may thereby increase the likelihood of findings of diminished or uninterpretable significance. In addition, when acute stress is a particular problem, any form of coerced activity may also contaminate the experimental finding with the stress often induced by any such procedure.

In the same fashion, simple manipulable objects, while generally suitable for most study subjects, rarely hold subjects' interests for prolonged periods (Watson *et al.*, 1989; Crockett *et al.*, 1989) and, in any case, may be precluded in studies involving motor function by competing with the specific dependent measures of the study; manipulable objects to which waste matter may cling or become embedded also must be subjected to frequent sterilization if they are not to serve as conduits for diseases which are antithetical to the basic purposes of any given study. Finally, as one additional example of the problems inherent in proposing any fixed, cross-paradigm regimen for enrichment, those studies involving indwelling devices for the administration or collection of biological, electrical, or pharmacological materials pose special problems regarding methods of enhancement. In particular, such studies may require a degree of restraint which for the safety of the subject, as well as the

purposes of the experiment, must preclude intersubject manipulation, gross movements, or contact with manipulanda which might destroy or interfere with these often delicate physiological interventions. Even during required periods of removal from restraint, which should be incorporated when not in conflict with research goals, indwelling devices and recording connections can significantly constrain, though by no means completely preclude, the possibilities of social and physical environment enrichment.

III. SIGNIFICANT DIMENSIONS OF ENRICHMENT

Based on the integration of the issues discussed previously, a variety of established research findings, and research which is currently underway, we would like to propose several major dimensions of environmental enrichment which should be considered for incorporation within any objectively based scheme designed to enhance psychological well-being. While specifics cannot be predicted reliably, it is our view that, new developments notwithstanding, any future programs seeking to materially enhance psychological well-being should incorporate significant reflections of most, if not all, of the environmental dimensions outlined next.

A. Subject Control of Salient Environmental Events

In light of many of the issues discussed earlier regarding the inability of humans to subjectively assess the perceptual and preferential world of the nonhuman primate, one approach to determining which aspects of the environment best suit subjects of different species, and of different classes within species, is to allow subjects to determine these dimensions themselves (see critique of these methods, Curtis, 1985). Simple lever devices, connected to relatively inexpensive solid state control systems, can allow subjects to control illumination levels and their circadian or other rhythmic changes, to alter temperature in the same way, and even to control when and if to interact with social partners of various types. More sophisticated programs might also allow control over auditory stimulation and even the appearance of various perch sites through similar subject-control devices. Such procedures not only turn over to the subjects the research task of determining preferred environmental features, but the technique itself actively engages the subject in forms of manipulation of the environment in which they are less likely to grow bored over time.

B. Attention, Work, and Strategic Efforts to Obtain Daily Rations

In addition to providing subjects the opportunity to generate or modify key dimensions of their environment, subjects can also be induced to remain alert, attend to and discriminate between relevant stimuli, and exert varying forms of physical effort to obtain desired incentives through a variety of methods. Perhaps most significantly, studies of "closed economies," i.e., ones in which requirements and resources are fixed (Collier, 1983), suggest that such techniques may be used to encourage subjects to engage their environments more fully by generating psychologically complex "strategies" (i.e., more beneficial cost-to-benefit ratios) for meeting the total demand of the environment in which they live. It may reasonably be assumed that the benefits of achieving maximization of efficiency characterize a significant part of natural selection pressures.

Mere time restrictions on feeding and/or watering do not suffice to encourage subjects to engage their environments more fully; under these conditions subjects may remain lethargic during most of the day and hyperaroused during the brief intervals of incentive presentation. Furthermore, in social settings, the stress and, for some, the physical danger of competing for the available food may more than undo any stimulative benefits. In contrast, fairly simple methods may be employed to engage the subjects more fully through the day, while still permitting subjects to maintain the intermittent rhythm of consumption that they may prefer. Thus, food or water may become available for brief periods during the day but at different locations, randomly distributed either by hand or through mechanical means. In such instances subjects must move through their environment when they seek these incentives, repeatedly checking the possible sources. Similarly, one or more sites on opposing walls of the cage or at floor and shelf levels, for example, may be signaled by means of a cue light for brief periods to indicate that a given device is available for inspection; under such conditions, subjects must maintain moderate levels of vigilance by frequently scanning their environment for the sign for incentive availability. Either of these techniques alone or in combination should require the animals, when motivated, to maintain attention and be prepared to move rapidly to that part of the environment in which the desired incentive is briefly available.

Based on a variety of work in our own and other laboratories (Rosenblum and Smiley, 1984; Bloomstrand *et al.*, 1986; Chamove *et al.*, 1982; Boccia, 1989; Bloomsmith *et al.*, 1988), more elaborate and engaging methods of eliciting foraging behavior throughout delimited or broad parts of the day can be employed. Food items can be secreted in various locations within the environment such that sufficient food for maintenance and growth is always available while its location is obscure. In such circumstances, when animals are required to search their environment for varying periods or exert varying levels of energy to obtain their food, subjects of many species remain engaged for prolonged daily periods over virtually indefinite periods of captive maintenance. Any such approach, to avoid adding stress rather than alleviating boredom, must be initially finely tuned to the species' known perceptual, motoric, and cognitive capacities and must be adjusted as these capaci-

ties further reveal themselves. Successful methods for sustaining attention and interest for prolonged periods include standard chow buried in containers (or in pen areas not likely to be soiled), with varying degrees of restricted visible access; devices which require a degree of puzzle manipulation to gain access to food; portable multiple-site foraging devices from which food must be extracted with considerable care and diligence; and operant systems in which subjects may gain access to food by completing fixed or variable interval or fixed or variable ratio reinforcement schedules. Obviously, when the housing arrangement allows, those foraging approaches which require spatial dispersal of food elicit active locomotor output as well as psychological engagement, whereas simple stationary operant procedures may actually deter movement through the captive field. As further evidence of the "investment" primate subjects make in these foraging efforts, it is worth noting that, unlike *ad libitum* feeding situations in which as much as 50% of acquired food may be wasted, when subjects "work" (i.e., expend considerable time and energy) to obtain their daily rations, they consume virtually all food that is found; depending on colony size, the saving in wasted food could easily pay for the initial cost of the equipment or manpower needed to implement these foraging enrichment approaches.

A somewhat more sophisticated, but still relatively "low-tech," procedure for feeding control was developed originally by Collier (1983) with a number of different species and employed in our laboratory with group- and singly-housed primates (Paully, 1984). With this method, two steps are involved in obtaining food, which seek to emulate the search and acquisition aspects of natural foraging. Subjects must engage in an initial operant task (the "search" phase) which, when completed, activates a second operant procedure; it is completion of this second task which actually produces the food rewards (the "acquisition" phase). The two phases may involve two adjacent operant levers, for example, or could incorporate two different operant tasks (e.g., a wheel turning and lever combination). In this technique, once food is "found" via the first procedure (the "search"), subjects can obtain as much food as they want via the second ("acquisition") operant, until they leave the food site. Upon their return to the food site, they must begin the initial "search" phase again. By manipulating the relative costs of the search and acquisition phases of the sequence (e.g., the number of lever presses required), one can alter the pattern of "meals" which subjects choose to take throughout the day. As the search costs go up, most subjects spontaneously reduce the number of meals they take each day, increasing the size of meals accordingly. By this means the subjects engage in a strategy which improves the relative cost-to-benefit ratio of feeding. This strategic adjustment can be shifted as the costs of search and acquisition are manipulated across a period of days. The method not only sustains subject engagement quite reliably, but the psychological or cognitive reassessment of the changing payoff of their foraging tasks would appear to represent an important form of sustained psychological enhancement.

C. Variation in Physical Environment

In keeping with the likely beneficial effects of a nonstatic housing environment, although as yet lacking empirical verification, there may well be some salutary stimulative effects of an environment containing several salient elements which gradually change over time. Light–dark ratios, intensity of illumination, thermal changes, and even auditory and olfactory cues can be altered over the course of days or weeks. If control of some of these environmental characteristics has been turned over to the subjects, we may further enhance or sustain the interest provoked by subject-controlled, preference procedures by altering the range of stimulus change within which manipulations can be made, e.g., the levels of illumination which the subject can generate.

Unlike the effects of acute alterations of minor aspects of the environment (e.g., the presentation of video images) which might be psychologically enhancing, sudden, massive, and unpredictable alterations of the environment are likely to be stressful. But benefits can be achieved through gradual change procedures which, though minimizing stress, place a continuing psychological demand on subjects to adapt various aspects of their daily activity, feeding, and interactive patterns. As is true of most enrichment procedures, for beneficial rather than detrimental psychological effects to be achieved, subjects must have the capacity to cope with changes rather than being periodically overwhelmed by them. A variety of studies have shown that both a loss of control over critical environmental features and a lack of predictability about them are typically quite stressful for primates and should be avoided in efforts at environmental enrichment (Mineka and Hendersen, 1985; Rodin, 1986; Mineka *et al.*, 1986; Hanson *et al.*, 1976; Snowdon and Savage, 1989).

D. Conspecific Associations

Most of those who have worked with many species of primates agree that in general those primates that are social in nature appear to benefit from regular opportunities for social interaction with conspecifics. Recent research has suggested that even simple, single-partner pairings appear to enhance the well-being of certain animals (Reinhardt *et al.*, 1988). As discussed earlier, however, considerable care must be taken in establishing these social opportunities. Not all species are gregarious, and although even the less social species may accept social partners, in the absence of clear data on adaptation and long-term stress of one or more of the members of these artificially formed groups, we must remain cautious regarding the overzealous application of this means of "enrichment." Similarly, males and females of a given species are often not equally accepting of social partners of either their own or the opposite sex (Coe and Rosenblum, 1974). Some species, such as the pigtail macaque, can be quite gregarious with members of their own consanguinal kinship group, but are relatively hostile

toward nonkin (Rosenblum, 1971). Finally, subjects younger and smaller than other group members and aged, injured, or debilitated subjects may find the stress of subordination or dominance contention present in inappropriate group or partner settings far too stressful to make such housing desirable. In conditions in which such social experiences are intermittent, these same negative social factors are likely to be considerably exacerbated (Kaplan *et al.*, 1983; Woolverton *et al.*, 1989). Given the frequent physical health benefits of individual housing for many individuals, social housing or periodic socialization should be employed cautiously until clear empirical evidence of both short- and long-term consequences of such experiences are available for different species and classes of subjects within species.

Although, for a number of the reasons discussed in sections earlier, not all subjects can, or perhaps should, be exposed to direct contact with conspecifics, it is possible that some of the salutary effects of social stimulation may be obtained without direct contact between animals. Various levels of interaction can and do occur through mesh and glass barriers while preserving safety for all subjects. In addition, when even this degree of contact is not possible, there is good evidence that primates of various species will spend long periods watching other animals via video; subjects will, in fact, operate various operant devices for the opportunity to observe another subject of their species, will show sustained preferences for viewing familiar partners, and will display socially relevant responses to video image communications (Bloomsmith *et al.*, 1990; Rosenblum and Paully, 1980; Plimpton *et al.*, 1981).

E. Future Directions for Cognitive and Motor Enrichment

It is perhaps fortuitous that the growing concern with the desirability of producing psychologically enriched environments for primate subjects has coincided with the advent of relatively inexpensive yet powerful computers. Developments by Rumbaugh and co-workers (Hopkins *et al.*, 1989; Washburn *et al.*, 1989; Richardson *et al.*, 1990) have wedded this computer technology with color video and related solid state technology to produce a range of tasks presented to the subject on a color monitor, in which solutions to the tasks are achieved through the manipulation of a video game "joy-stick." Movement of the cursor into contact with the correct target produces a small treat for the subject. Problems involving simple movement and the striking and/or "capture" of a moving target, as well as increasingly complex learning and discrimination problems, have been employed successfully with this paradigm. Observations over extended periods of many months indicate that subjects will engage in many hundreds of these "games" each day, at times not even removing all the pellet rewards. As testimony to the inherent interest these games appear to have, our own data make it clear that the subjects will generally maintain relatively high investment in these games even though regular food rations are readily available. Thus, as is generally most desirable, subjects are not coerced, but choose to engage these enrichment devices. Research by the current authors has used these video game devices in group-housed macaque groups, including a group of mothers with young infants. By reducing the video screen size we have demonstrated the usefulness of this technique with squirrel monkeys in a social group as well as singly housed. As with all electronic equipment maintained under laboratory conditions, these devices require some periodic repair. But in our laboratory, with a total of 13 video game devices in constant use, daily food loading, data recording (if research is underway), and machine checkup and cleaning require less than 2 hr of work per day; with extensions to food-hoppers, weekend loading can be avoided and other skip-day maintenance schedules can be instituted. Research is currently underway to establish the characteristic patterns of use of these computer–video devices in various types of subjects over long periods of time; their effectiveness in deterring the development of and in ameliorating existing levels of positive and negative symptom pathology also will be explored. Our current data, compiled over nearly 4 years of use, indicates that individual subjects employ the machines in several bursts of activity during the day. As a consequence, a further study of the efficacy of intermittent as well as constant access to these systems will determine the economic costs and procedural feasibility of employing these devices in large, active laboratory settings. There is good reason to believe that when economically feasible, and when their use does not interfere directly with experimental procedures, these relatively benign computer–video devices may prove invaluable in our future efforts to enrich, in a psychologically meaningful way, the environments of both singly and group-housed subjects of many different species.

IV. SUMMARY

There are several central themes in our perspective on environmental enrichment. It is imperative that we recognize the inherent limitations in the use of human, a priori judgments regarding the nature of environments most desirable for a wide range of primate species maintained under laboratory conditions. In addition, we must develop a body of hard data regarding the preferences of species and classes within species for different environmental components. We also must assess empirically the actual effects that ensue when those conditions are imposed; in keeping with the current "performance standards" approach, the short- and long-term dysfunctions which do or do not actually emerge as either positive or negative symptoms should be used as the ultimate criteria by which the suitability of a given setting is judged in relation to the research objectives being pursued. Finally, we suggest that one environmental feature of particular importance is the degree to which animals can exercise overt control over various aspects of their environ-

ment. New electronic devices and programming abilities offer the possibility of significant new advances in this area. It is clear that with careful attention to the demonstrable needs of different types of subjects we can fully realize our significant research goals while maintaining our primate subjects under conditions which maximize their health and well-being.

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CHAPTER 7

Primate Conservation

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I. INTRODUCTION

Scientists who choose to conduct laboratory research involving nonhuman primates must become very knowledgeable about primates. This knowledge should range from the specific details of comparative primate biology that are directly relevant to the research topic and to awareness of the natural history, geographic distribution, social organization, dietary adaptations, and conservation status of wild populations of the species employed. This knowledge is essential for scientists to choose the most appropriate species for each research project and to explain those choices to animal care and use committees, scientific peers, funding agencies, and the public.

An understanding of the conservation status of wild populations is essential to scientists and funding agencies to assure that the species will be available for future use and to assure that research use does not contribute to declines in natural populations. The availability of a species for research use increasingly requires attention to conservation and animal welfare

† Deceased

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issues, and opportunities exist for all biomedical users of primates to make positive contributions in these areas. Changes in wild primate populations can result in fluctuations in availability and cost. These factors are primary influences on species choice for research. The best interests of the biomedical research community are served by funding and participation in objective population studies of wild primates and maintenance of an understanding of conservation issues in source countries.

Facility and personnel requirements differ according to species; consequently, primate housing and care systems must be designed to fit the species that is to be studied (e.g., the requirements of chimpanzees, macaques, and marmosets differ substantially). Within some boundaries, facilities can be purposely designed to allow flexibility with regard to species choice.

The legal requirements for importation, use, and transportation within and between countries or states can change quickly in response to real or perceived changes in the conservation status of wild populations. Efforts are underway in the United States and Europe to eliminate any importation of nonhuman primates for research use. Those who philosophically oppose the intervention of humans in the lives of any animal (and thus oppose all primate research) argue successfully that wild populations are being depleted. Objective studies are needed to monitor populations and provide the basic evidence upon which rational decisions can be made. Biomedical scientists can have an advantage in such debates if they are well informed on primate population issues.

As with other valuable and limited resources, the conservation of primates may require the following actions: (1) management of wild populations through cropping, relocation, or reintroduction from captive stock; (2) reduction, temporary suspension, or elimination of capture from wild populations; (3) reduction of wastage during capture, transport, and conditioning through use of more humane methods, facilities, and care; (4) increased captive propagation in source countries and/or use countries; (5) reduction in use rates; (6) limitation of use to highest priority projects; (7) more efficient use of each individual animal (e.g., by use in many studies sequentially); (8) increased use of information from computerized databases from captive colonies; (9) use of more efficient experimental designs or more powerful statistical techniques; (10) development and use of specialized breeds or strains of primates for specific uses; (11) increased recognition of the value of deviant individuals and use of such individuals as natural models; (12) efficient use of captive breeding stock; and (13) replacement of one species by another or by some other alternative. For example, one nonhuman primate species might be replaced in a biomedical research program with a more plentiful closely related species or with humans (e.g., human clinical trials may be warranted sooner than usual in some cases), nonprimate animals, or nonanimal alternatives.

Reduction, refinement, and replacement can be valid approaches to primate conservation; however, they can be counterproductive if wrongly applied. Many aspects of comparative primate biology and behavior in illness and in health have not been adequately studied, so an overall reduction in primate research would not be consistent with obtaining valuable knowledge that may prove critical to the development of future treatments for human disease. Just as the knowledge gained from scientific research involving primates is vital to progress in the human health sciences, valid information is essential to the health and well-being of nonhuman primates and to conservation of the ecosystems in which they participate.

The major issues involved in the conservation of nonhuman primates are introduced here. Practical information on methods of conserving nonhuman primates is also provided. The ultimate goal of this chapter is the facilitation of efficient, high quality scientific research conducted in ways that promote the humane care and use of primates and benefit human and wild primate populations.

The relevant published literature is reviewed, although not exhaustively, and the authors have relied on their very substantial experience in biomedical and behavioral research laboratories, breeding colonies, zoological parks, and natural settings. Some of the most important issues were reviewed in "Primate Conservation in the Tropical Rain Forest" (Marsh and Mittermeier, 1987). Mittermeier *et al.* (1986) also provided a substantive review of the issues in "Comparative Primate Biology: Behavior, Conservation, and Ecology" (Mitchell and Erwin, 1986, pp. 3–72). "Primates: The Road to Self-Sustaining Populations" (Benirschke, 1986) includes an array of contributions regarding virtually all aspects of primate conservation. Another excellent source of information relevant to primate conservation is "Primate Societies" (Smuts *et al.*, 1986), especially the chapter by Mittermeier and Cheney (1986, pp. 477–490). A detailed assessment of the ranges and conservation status of all primate species is included in "Primates of the World: Distribution, Abundance, and Conservation" (Wolfheim, 1983). Earlier contributions included "Primate Conservation" (Prince Ranier and Bourne, 1977) and "Primate Utilization and Conservation" (Bermant and Lindburg, 1975), which provided insight on the extraordinarily sophisticated plans and activities regarding primate conservation, field research, and biomedical use of primates. Many of the recommendations made here are similar to those identified in the 1970s (Southwick and Blood, 1979).

Some of the conservation issues discussed in this chapter are matters of direct concern with regard to wild populations. Habitat loss, hunting, trapping, feeding ecology, nutrition, reproduction, diseases, predation, zoonotic potential, and pest control are all relevant to the conservation of wild primate populations. Other issues are addressed because of their direct relevance to primates residing in laboratories, breeding colonies, and zoological parks and to the conduct of scientific research in those settings. Among the most important of these issues is the number of animals involved, the origins of the animals, the role of captive propagation programs and reproductive technologies, the quality of health care, the quality of life, the quality and priority of the research, and the disposition of animals that are

surplus to research needs or captive breeding programs. The issues regarding wild and captive primates are not mutually exclusive, and this chapter is intended to clarify some possible relationships among these sets of issues.

II. PRIMARY THREATS TO WILD PRIMATES

The major threats to wild primate populations are habitat destruction, hunting for food, eradication as agricultural pests, and live capture. There is no doubt among well-informed conservation biologists that habitat destruction is by far the most serious of all threats to primate populations and the ecosystems in which they reside (cf. Erwin, 1982, 1986; Mittermeier and Cheney, 1986; Mittermeier *et al.*, 1986; Myers, 1987, Southwick *et al.* 1986). The impact of live capture for biomedical research is usually insignificant in comparison with other influences on wild populations. An exception has been the rhesus monkey which was excessively trapped for export in the 1950s and 1960s. Subsequent bans on exportation resulted in remarkable population growth (Southwick & Siddiqi, 1988). Of course, the impact of capture for biomedical use can be greatly reduced if sustained-yield cropping is practiced in coordination with wildlife management and captive propagation programs.

A. Habitat Loss

The vast majority of nonhuman primate species (but not all) live in tropical rain forests (Marsh and Mittermeier, 1987). These forests are being cut at an alarming rate, and once the forests are destroyed they seldom can be restored. The loss of forests means that the wildlife populations residing there are usually eliminated and cannot be reintroduced.

Estimates of global tropical forest deforestation rates (that is, *elimination* of forests) range from about 70,000 square km (>25,000 square miles) to more than 90,000 square km (>35,000 square miles) per year (Myers, 1987). An area about the size of the state of Maryland is deforested every 3 months. An area larger than the state of Washington is destroyed or degraded every year.

The ultimate causes of habitat destruction are poverty and the rapid growth of human populations in many tropical forest resource countries, along with the demand by industrialized countries that consume more forest products than they produce. Conversion of savannah habitat to grazing land or other agricultural use also displaces primate populations. Conflicts between human and nonhuman primates are inevitable as human populations grow. The world's human population grew by at least 100,000,000 during 1992, with much of this growth occurring in the developing countries in which wild primate populations reside.

Campaigns in industrialized nations against the use of tropical forest products are plausibly viewed by authorities in developing countries as inconsistent with past (and present) forest use practices in the industrialized countries, but tropical forests are not cut just to gain income from forest products; forests are also removed to convert land to agricultural use. Clearing occurs in many places even when there is no market for the timber. The jobs, roads, and cleared land that accompany logging operations increase settlement of rural areas. Conversion of forests or savannahs to monoculture plantations of oil palm, teak, tapioca, cardamom, cloves, eucalyptus, etc. has had mixed effects. The growth of eucalyptus for firewood, other products, and erosion control has had some long-term benefits. Teak culture has sometimes replaced the removal of this species from older forests. However, biological diversity and carrying capacity are diminished by the development of monocultural plantations and, in some cases, the economic gains are short-lived. For example, the value of oil palm and clove plantations relative to the forests they replaced has declined along with the demand for palm oil and cloves.

Agricultural uses of previously forested areas include shifting subsistence farming, cattle ranching, and intensive farming to produce food or other products for domestic use or export. Shifting agriculture may be the most devastating problem because the practice often results in degradation of land to the point that it is no longer useful for growing crops due to erosion and loss of fertile soil. In 1978 it was estimated that more than 250,000,000 people in tropical areas practiced shifting forest farming (UNESCO, 1978).

Shifting cultivation is apparently the primary means by which primary moist tropical forest is converted to secondary forest (Marsh *et al.*, 1987). Although many primate populations are virtually unable to survive outside primary forests (e.g., gibbons), others (notably rhesus and cynomolgus macaques) thrive in secondary forests and in commensal relationships with humans (Southwick and Cadigan, 1972; Wilson and Wilson, 1975).

In many tropical areas people rely on firewood or charcoal as their primary energy resource for cooking and/or heating. In addition to use in rural areas near forests, firewood and charcoal are transported into urban areas for home and industrial use. In some primate endemic areas such as Madagascar, deforestation due to gathering of firewood has already devastated large areas and may soon eliminate the habitats of some primate populations (Richard and Sussman, 1987).

B. Hunting of Primates for Food

Although habitat loss is clearly the most significant and widespread threat to primate populations, hunting of primates for use as food is common in many areas where wild primates exist. The impact of hunting on primate populations has been reviewed by Mittermeier (1987), and he concluded that "hunting can cause more damage than forest destruction in certain regions and has already resulted in the loss of local populations

even in areas of otherwise suitable habitat" (p. 109). Local subsistence hunting does not usually pose a serious problem if human population densities are low and an abundant primate habitat is available; however, commercial or market hunting can be devastating.

Commercial market hunting for primates is common in central and west Africa, parts of Central and South America, and in some parts of Asia. In Africa the most hunted primates appear to be mangabeys, guenons, mandrills and drills, and sometimes gorillas. Studies of "bush meat" preferences have indicated that primates are highly prized. Smoked primate carcasses are transported long distances by the truckload for sale at market in west Africa. Woolly monkeys (*Lagothrix*) and spider monkeys (*Ateles*) seem to be the most popular primates sold for food in Latin America, and local populations have been apparently hunted to extinction in some cases (Mittermeier *et al.*, 1986). Hunting of primates for food is less widespread in Asia than in Africa and Latin America. Where it is practiced it is usually limited to aboriginal tribes or ethnic minorities. Even so, hunting appears to be largely responsible for the decline of some species. For example, the crested black macaque (*Macaca nigra*) of North Sulawesi, Indonesia, is now seriously threatened by commercial hunting. In the Tangkoko Nature Reserve in which the population had been estimated at >25,000 in 1978, the population had declined to less than 2500 by 1987 (Sugardjito *et al.*, 1989).

Religious and ethnic customs influence the inclusion of nonhuman primates in human diets. Little human predation on nonhuman primates occurs in predominantly Muslim or Hindu regions or Africa and Asia, whereas monkey eating is relatively common in some predominantly Christian and animist areas and in some ethnic communities. Primate flesh and other body parts are used in some cultures for medicinal, aphrodisiac, or other ceremonial purposes. A few primate species have been hunted for their skins, notably the black and white *Colobus* sp. of Africa, the golden monkeys (*Rhinopithecus* sp.) of China, and the golden lion tamarin (*Leontopithecus* sp.) of Brazil. These skins have been used ceremonially and for coats and hats, sometimes for export. *Colobus* is less commonly eaten than other primate species, but the use of many other primate skins or body parts appears to be secondary to hunting for food.

C. Extermination as Agricultural Pests

In some areas, primates are serious agricultural pests. They raid gardens and cash crop plantations and can quickly ruin what has taken subsistence farmers much time and labor to produce. It is not surprising that the farmers in such areas retaliate by chasing, clubbing, shooting, snaring, or poisoning the primates with whom they come into direct conflict. Efforts to conserve primates must include evaluation of the effects of primates on agriculture. Only those programs that effectively address the needs of local people for relief from crop raiding have realistic prospects of success. The primates most often mentioned as crop raiders are the mangabeys, baboons, vervets, and various other guenons of Africa and the macaques of Asia. Corn (maize), papaya, and cacao are among the most frequent targets of crop-raiding primates. These are also among the first crops planted by shifting agriculturalists and farmers cultivating newly deforested areas. Macaques and mangabeys seem to be especially attracted to corn and cacao, and both are known to leave a high quality habitat to raid these crops. Local common names of some *Cebus* species reflect their known habit of raiding corn gardens (Mittermeier, 1987). Guenons introduced into the Caribbean (St. Kitts and Barbados) and macaques introduced on Mauritius and elsewhere are notorious crop raiders. Introduced primate species also endanger endemic species. The long-tailed macaques (*Macaca fascicularis*) of Mauritius pose a serious threat to several rare species of birds (cf. Mountfort and Arlott, 1990).

In some of the areas where crop raiding has become a serious problem, systematic extermination efforts have been carried out, often through bounty systems. Mittermeier (1987) reports that such a campaign was conducted in Sierra Leone between 1947 and 1962, with government documentation of about 245,000 primates destroyed during that interval. Bounty programs have also been mounted in the Caribbean. Clearly, as human populations continue to increase and tropical forests are converted to agricultural use, conflicts between human and nonhuman primates will further escalate. The effective use of wildlife management techniques to control wild primate populations in conflict with humans presents difficult choices, but the challenge must be met. In most cases, capture and relocation to an available habitat or capture for biomedical supply is the only realistic alternative to programs of outright extermination, although learned "taste aversion" using sublethal doses of lithium chloride has been attempted with baboons and may be feasible in some settings (Forthman Quick, 1986).

Extermination of primates as agricultural pests has sometimes been integrated with the commercial food market supply. A double incentive exists for extermination of those species that are simultaneously abhorred as pests and preferred as food. Primates regarded as pests are also captured alive, usually as infants, and are kept as pets or for sale.

D. Live Capture

The live capture of nonhuman primates has occurred for thousands of years for a variety of purposes. The largest volume of capture was of rhesus monkeys in India, especially for the development and testing of the polio vaccine. During the height of importation of rhesus monkeys in the 1950s, between 100,000 and 200,000 were exported annually. By 1965 the rate of export had declined to about 50,000 per year, and by 1975 to 20,000. A total ban of rhesus exports from India was implemented in 1978. Populations of rhesus monkeys in India have been monitored for more than 30 years (Southwick *et al.*, 1961a,b), and the effects of harvesting and banning of export are reflected in the results of this

program (Southwick and Siddiqi, 1988). Southwick *et al.* (1970) estimated an annual birth rate of about 176,000 rhesus monkeys in Uttar Pradesh province alone and suggested that 60,000 rhesus monkeys could have been harvested annually with no serious consequences for the wild population if no other ecological forces were acting against the rhesus population. Unfortunately, several other forces were depressing primate populations in India at the same time; notably, habitat losses and changing attitudes of Indian villagers, so that removal of even 25,000 rhesus per year represented an unacceptable loss due to trapping and export.

A sustained-yield management program could have conserved self-sustaining healthy rhesus populations, reduced crop damage and other human–macaque conflicts, promoted a secure supply of primates for biomedical use, and provided needed economic sustenance for local people. Neither uncontrolled trapping nor an export ban was compatible with this approach. Rhesus populations declined prior to the export ban and partially recovered after the ban was implemented. Now the burgeoning rhesus populations in some areas have led to renewed calls for eradication.

This problem points out the need for careful integration of wildlife management and primate supply programs. The export ban is not effective as a long-term conservation strategy and may ultimately damage primate conservation efforts in India. The attitude that primates are agricultural pests, marketplace nuisances, and health hazards is becoming prevalent in some areas as a result of unchecked population growth of rhesus populations and increased competition with humans for limited resources. Programs that advocate either uncontrolled trapping or total bans on removal threaten sustainable primate conservation.

III. PATTERNS OF PRIMATE USE

Any discussion of conservation in relation to the use of primates in biomedical and behavioral research must describe the patterns of use, including some information on the species and numbers used, the origins and disposition of the primates studied, and the primary disciplines and research areas in which primates are used. Information of this kind is essential to an understanding of the prospects and desirability of developing alternatives to the study of nonhuman primates. The relevance of conservation concerns must also be addressed in the context of primate use patterns. For example, if studies are conducted without the removal of primates from the wild (e.g., use of captive bred animals, capture and release techniques, or noninvasive observation) or if wild primates that would otherwise be exterminated as pests are captured, there is little prospect that research use will damage wild populations.

A. Species Studied

One way of estimating which species are most frequently involved in research is to count the number of research publications based on work done with various primate species. Erwin and Zucker (1987) analyzed citations in the outstanding bibliographic resource "Current Primate References" for the years 1980–1984. They limited their study to Old World monkeys. The four most cited species for each of the 5 years examined were *Macaca mulatta, Macaca fascicularis, Papio cynocephalus*, and *Cercopithecus aethiops*. An examination of some recent issues (1990) of *Current Primate References* revealed that *M. mulatta* is still by far the most commonly studied primate species and that *M. fascicularis* is the second most commonly studied. The African green monkey (*C. aethiops*) and various kinds of baboons, especially *P. cynocephalus*, continue to be popular with researchers. Other macaques (*nemestrina, fuscata, arctoides*, and *radiata*) are among the Old World monkeys most frequently involved in research along with *Erythrocebus patas*. As expected, chimpanzees are the most frequently studied of the great apes. Squirrel monkeys (*Saimiri* sp.), owl monkeys (*Aotus* sp.), and common marmosets (*Callithrix jacchus*) are the most studied New World monkeys. More than 28% of the citations in two recent (1990) issues of *Current Primate References* dealt with rhesus macaques and more than 18% with cynomolgus macaques. Clearly, a very small number of species (two) accounts for a very large proportion (46%) of the scientific research that involves nonhuman primates.

Most rhesus monkeys and all chimpanzees currently entering research use in the United States are captive bred. Increasing numbers of cynomolgus macaques are purposely bred in countries of origin. Domestic breeding of this species is also increasing in the United States, and importation of wild-caught primates into the United States and Europe may soon end altogether, although Japan is unlikely to impose any ban on importation of wild-caught primates. Sustained-yield harvesting is already practiced with several species, from naturally occurring populations (e.g., *Saguinus, Saimiri*, and *Aotus* from Peru), introduced populations (e.g., *C. aethiops* from St. Kitts and Barbados; *M. fascicularis* from Mauritius), or island breeding programs (*M. fascicularis* from Indonesia and Philippines; *M. mulatta* from Puerto Rico).

B. Types of Use

Estimates of primate use are often presented as if all studies of primates involved killing of the animals studied. Although some studies of nonhuman primates are terminal, many are not. Studies of reproductive biology and behavior seldom involve killing the animal at the end of the study if the animal is a nonhuman primate. Many neuroscience or toxicology studies include humane euthanasia as a part of the research protocol because these studies require careful necropsy with measurement of site-specific effects or perfusion and preparation of slides to evaluate sites of experimental intervention.

In many communicable disease studies the primates involved do not become ill nor are they killed. This is true, for example, with chimpanzees in human immunodeficiency virus (HIV) and

hepatitis research and with most vaccine testing. However, objections have been set forth against such studies on the grounds that individual caging and quarantine isolation constitute inhumane treatment. These objections have been met by environmental enrichment programs and development of innovative biocontainment environments (Erwin and Landon, 1992).

The degree to which primate use is relevant to conservation varies with the type of use and the discipline represented. Primate use by discipline or topic can be estimated by examination of publications dealing with primates. Erwin (1981a,b) reviewed research involving primates based on the number of publications on each topic in *Current Primate References* for 1980. Those results are compared in the tabulation presented next with the listings in a 1990 issue of *Current Primate References.*

1980	%	1990	%
Nervous system	13.4	Nervous system	14.8
Pharmacology	9.8	Virology	10.7
Behavior	9.2	Pharmacology	9.3
Dental/oral	5.2	Reproduction	8.6
Endocrinology	4.9	Colony management	6.2
Cardiovascular	4.8	Cardiovascular	5.6
Reproduction	4.6	Endocrinology	5.2
Environmental health	4.1	Behavior	4.3
General primatology	4.0	Biochemistry	4.1
Nutrition	3.4	Immunology	3.6

Nearly twice as many publications dealing with primates were listed in 1990 as were listed in 1980. The percentage of this total rose slightly for neuroscience, cardiovascular, and endocrine research, and declined slightly for research in pharmacology. Predictably, publications regarding virology and immunology rose sharply due to the acquired immunodeficiency syndrome (AIDS) crisis, as did studies of reproductive biology and primate colony management. The percentage of studies of primate behavior, environmental health, and ecology/conservation (1980: 2.7%; 1990: 1.3%) declined to less than half their 1980 percentages, indicating an absolute decline in the number of publications in these areas as well as a decline relative to other fields.

Three of the topics most critical to conservation waned or failed to grow during the 1980s. Studies of primate behavior and ecology seldom "use up" (i.e., terminate) animals, whereas studies of toxicology and environmental health more often do. The decline may have resulted from a lack of scientific commitment to these topics or a lack of funding to support such work. Studies of reproductive biology and colony management have important conservation applications so the growth in the number of publications in these areas is a positive sign. The growth in neuroscience studies probably involves a substantial net increase in the number of primates studied, and this is also likely for cardiovascular and endocrine studies; however, most neuroscience studies involve very small numbers of primates. An examination of 16 scientific articles published in the *Journal of Comparative Neurology* during the first 3 months of 1991 revealed that 96 primates were used, an average of 6 primates per study. The most commonly used species was *M. fascicularis* (39%) followed by *M. nemestrina* (30%) and *M. mulatta* (8%). Other primates used were baboons (*Papio* sp.) (4%), squirrel monkeys (*Saimiri sciureus*) (8%), capuchins (*Cebus apella*) (6%), owl monkeys (*Aotus trivirgatus*) (1%), and bushbabies (*Galago crassicaudatus*) (3%). This sample suggests that three macaque species account for more than 75% of the neuroscience work currently involving nonhuman primates. If 6 is the typical number of primates used in neuroscience studies and if about 950 neuroscience studies involving nonhuman primates were published in 1990, about 5700 primates would have been used in neuroscience studies published in 1990. Approximately 2200 of these would have been *M. fascicularis*, mostly wild caught in Indonesia, the Philippines, or Mauritius. Most other macaques in neuroscience studies would have been captive bred in the United States.

IV. PRIMATE POPULATION STATUS

Efforts have been underway for some time to determine which primate species are most in need of conservation action. An understanding of primate population status is also essential to regulation and enforcement of the Convention for International Trade in Endangered Species (CITES) and the U.S. Endangered Species Act (ESA), planning of primate supply for scientific use, and determination of priorities for captive breeding programs.

Beginning in the early 1970s, a major study was conducted regarding the distribution, abundance, and conservation status of primates (Wolfheim, 1983). This landmark work was funded under a contract from the U.S. Fish and Wildlife Service (USFWS) to the World Wildlife Fund (United States) and the resulting volume is important for reference, although the information is no longer current due to changes in the status of many primate populations.

The Primate Specialist Group (PSG) of the Species Survival Commission (SSC) of the International Union for the Conservation of Nature and Natural Resources (IUCN) has been a leader in developing "Action Plans for Primate Conservation." The Chairman of the PSG, Dr. Russell Mittermeier, has played a major role in the initiation of these plans and has prepared a model plan for the neotropical region (Mittermeier, 1987). Similar plans have been prepared for Africa (Oates, 1986; Oates *et al.*, 1987) and Asia (Marsh, 1987; Eudey, 1987), and some plans for specific countries have been prepared (e.g., Madagascar: Richard and Sussman, 1987) or are in progress (e.g., Indonesia).

A common theme that has emerged from attempts to determine the population status of various primate species is the lack of good census or survey data for most species. Nothing can

substitute for high quality survey data in determining population status, population trends, and conservation needs; but these data are difficult to obtain and generalizations from survey data can be risky. A Primate Population Monitoring Network (PPMN) has been proposed as a means of addressing the need for an objective population database for use in decisions about conservation, wildlife management, and action priorities.

A. Current Status of Primate Populations

The status of primate populations is codified in regulations, conservation lists, and action plans. These documents serve as the basis for developing conservation strategies, policies, and enforcement. Due to the dynamic nature of population problems and the difficulty of obtaining current and high-quality population data, these documents sometimes do not list species that are, in fact, in grave danger of extinction or may include species that are less vulnerable than the listing indicates. Some lists, such as the ESA list, undergo periodic review (every 5 years, e.g., 1987, 1992, etc.) and serve as the authority on which policy and enforcement are based.

One of the most authoritative lists is the "IUCN Red List" compiled by the World Conservation Monitoring Centre for the IUCN. The "1990 IUCN Red List of Threatened Animals" (International Conservation Monitoring Center, 1990) lists taxa as "endangered" (in immediate danger of extinction); "vulnerable" (expected to become endangered if current threats contine); "rare" (having small populations that are at risk); and "indeterminate" (thought to be at risk but available information is not adequate to assign status rating). Table I includes 63 species and subspecies of primates that are listed as "endangered." Seventy-six other taxa are listed as vulnerable, rare, or indeterminate. Only those listed as "endangered" are included in Table I due to spatial limitations and the relative urgency of conserving the "endangered" forms.

None of the most commonly studied primate species are listed as endangered, vulnerable, rare, or indeterminate; however, one subspecies each of three rather frequently studied species, the chimpanzee, Japanese macaque, and squirrel monkey, is listed as "endangered" in the "1990 IUCN Red List of Threatened Animals." The other two chimpanzee subspecies and the pygmy chimpanzee (or "bonobo") (*Pan paniscus*) are listed as "vulnerable."

The chimpanzee listing contrasts somewhat with that under the U.S. Endangered Species Act. A recent decision was made by the USFWS to regard all wild chimpanzees as "endangered" and the captive population as "vulnerable." The USFWS recognized that biomedical use of chimpanzees within the United States was no longer a threat to the wild populations because captive breeding is successful and can be expected to meet all future research needs. The "IUCN Red List" position seems to accurately reflect what is known about the actual status of wild chimpanzee populations. Other primate populations that are in great danger of extinction within their home range (e.g., the crested black macaque, *Macaca nigra*, of Sulawesi, Indonesia) are not included in the "IUCN Red List." A "Global Captive Action Plan for Primates" is in preparation under the direction of the Captive Breeding Specialist Group of the IUCN Species Survival Commission (Stevenson, Foose, and Baker, personal communication). The plan will include recommendations for Population and Habitat Viability Assessments (PHVA) and Conservation Management Plans (CMP). Clearly, the Primate Population Monitoring Network noted earlier would contribute to PHVA efforts.

The conservation status of primates under CITES Appendix I is shown in Table II. CITES went into effect in July 1975, as a means of regulating trade. CITES Appendix I lists "species threatened with extinction," whereas Appendix II lists "species not yet threatened but which could become endangered if trade is not controlled." Of course, any wild population could *theoretically* become endangered by excessive trade, so all nonhuman primates not already listed on CITES Appendix I or II were added to CITES Appendix II in 1977. Importation of any CITES Appendix I species now requires import and export permits, while any CITES Appendix II species requires an export permit from the country of origin.

Statutory authority for implementation and enforcement of CITES provisions resides with the Department of the Interior, U.S. Fish and Wildlife Service. The USFWS Office of Permits seeks the advice of the USFWS Office of Scientific Authority, which reviews all import permit applications. The USFWS also coordinates CITES and U.S. Endangered Species Act (ESA) provisions. Primates listed under the Endangered Species Act are shown in Table III.

The Endangered Species Act requires a permit to "take" any species listed as endangered. "Take" is defined to include capture, use, or transportation across state lines. Consequently, scientific studies involving species listed as endangered require permits, as do transfers from one zoological park to another. The permit process includes a requirement that applications for permits must be published in the "Federal Register" to allow for public comment. While this process seems appropriate for decisions about the importation of endangered species, it can be extremely cumbersome if applied to some captive populations. Campaigns to reclassify species as endangered sometimes are used to attempt to block or impede research use. For example, permits for importation of chimpanzees were already covered under CITES provisions when advocacy groups pressed to have the status of chimpanzees revised under the U.S. ESA. Imposition of a public comment period on each proposed research use was the apparent goal of the reclassification effort. Sadly, the misuse of the ESA to promote special interests has eroded support for the ESA and for conservation.

The method by which ESA listing decisions are made warrants some attention. Requests for listing (and resistance to listing) are often politically motivated. In such cases, the value of objective information becomes especially critical. The quality

TABLE I

NONHUMAN PRIMATES LISTED AS ENDANGERED IN "1990 IUCN LIST OF THREATENED ANIMALS"

Common name	Genus and species
Great apes	
West African chimpanzee	*Pan troglodytes verus*
Mountain gorilla	*Gorilla gorilla beringei*
Eastern lowland gorilla	*Gorilla gorilla graueri*
Orangutan	*Pongo pygmaeus*
Lesser apes	
Kloss's gibbon	*Hylobates klossi*
Javan gibbon	*Hylobates moloch*
Pileated gibbon	*Hylobates pileatus*
Old World monkeys	
Asian Cercopithecines	
Yakushima macaque	*Macaca fuscata yakui*
Mentawai macaque	*Macaca pagensis*
Lion-tailed macaque	*Macaca silenus*
African Ceropithecines	
Tana River mangabey	*Cercocebus galeritus galeritus*
Sanje-crested mangabey	*Cercocebus galeritus sanje*
Drill	*Mandrillus leucophaeus*
White-throated guenon[a]	*Cercopithecus erythrogaster*
White-throated guenon	*Cercopithecus erythrotis selateri*
Preuss's guenon	*Cercopithecus preussi*
Asian Colobines	
Javan leaf monkey	*Presbytis comata*
Mentawai leaf monkey	*Presbytis potenziani*
Tonkin leaf monkey	*Trachypithecus francoisi*
Nilgiri leaf monkey	*Trachypithecus geei*
White-headed black leaf monkey	*Trachypithecus leucocephalus*
Pig-tailed langur	*Simias concolor*
Red-shanked douc langur	*Pygathrix nemaeus*
Black-shanked douc langur	*Pygathrix nigripes*
Tonkin snub-nosed monkey	*Rhinopithecus avunculus*
Yunnan snub-nosed monkey	*Rhinopithecus bieti*
Guizhou snub-nosed monkey	*Rhinopithecus brelichi*
Sichuan golden snub-nosed monkey	*Rhinopithecus roxellanae*
African Colobines	
Black colobus	*Colobus satanus*
Red or bay colobus	*Procolobus badius* subspecies[b]
New World monkeys	
Cebids	
Wooly spider monkey	*Brachyteles arachnoides*
Masked titi monkey	*Callicebus personatus*
Southern bearded saki	*Chiropotes satanus satanus*
Yellow-tailed woolly monkey	*Lagothrix flavicauda*
Central American squirrel monkey	*Saimiri oerstedi*
Callitrichids	
Buffy tufted-eared marmoset	*Callithrix aurita*
Buffy-headed marmoset	*Callithrix flaviceps*
White-footed tamarin	*Saguinus leucopus*
Cotton-topped tamarin	*Saguinus oedipus oedipus*
Black-faced lion tamarin	*Leontopithecus caisara*
Golden-headed lion tamarin	*Leontopithecus chrysomelas*
Black lion tamarin	*Leontopithecus chrysopygus*
Golden lion tamarin	*Leontopithecus rosalia*
Tarsiers	
Tarsiidae	
Philippine tarsier	*Tarsius syrichta*
Prosimians	
Daubentoniidae	
Aye-aye	*Daubentonia madagascarensis*
Indriidae	
Indri	*Indri indri*
Diademed sifaka	*Propithecus diadema*
Golden-crowned sifaka	*Propithecus tattersalli*
Lemuridae	
Golden bamboo lemur	*Hapilemur aureus*
Greater bamboo gentle lemur	*Hapilemur simus*
Alaotran gentle lemur	*Hapilemur griseus alaotrensis*
Crowned lemur	*Lemur coronatus*
Sclater's lemur	*Lemur macaco flavifrons*
Mongoose lemur	*Lemur mongoz*
Ruffed lemur	*Varecia variegata*
Cheirogaleidae	
Hairy-eared dwarf lemur	*Allocebus trichotis*

[a]Should probably be listed as "red-bellied" guenon

[b]8 forms are listed as endangered, but taxonomy unclear as listed

of evidence on which requests or listings are based varies dramatically, but really comprehensive population studies are seldom available. Despite their scarcity, there is no acceptable alternative to systematic and detailed population studies.

Wild primate populations can seldom be exhaustively monitored because of population size, population range, and characteristics of the terrain and habitat. In most cases, surveys are conducted in sampled portions of a population's range, and the data from these samples and from habitat and range characteristics are used to calculate densities and to estimate the abundance of wild primates (Southwick *et al.*, 1961a,b). Poorly drawn or otherwise inadequate samples can provide misleading estimates. Consequently, the methods used in population monitoring must be carefully chosen and objectively applied.

The random sampling that would be ideal for accurate estimates of abundance can seldom be accomplished due to differential accessibility across population ranges. Densities of remote and relatively undisturbed portions of the population may provide the most representative information for estimating the total population, but available survey or census data are usually drawn from areas studied because primates are known to be abundant and accessible there. Surveys and censuses of re-

TABLE II

NONHUMAN PRIMATES LISTED IN CITES APPENDIX I.[a]

Common name	Genus and species
Great apes	All species
Lesser apes	All species
Old World monkeys	
Asian Cercopithecines	
Lion-tailed macaque	*Macaca silenus*
Asian Colobines	
Pagai Island langur	*Nasalis* (=*Simias*) *concolor*
Proboscus monkey	*N. larvatus*
Gray langur	*Presbytis entellus*
Golden langur	*P. geei*
Capped langur	*P. pilleata*
Mentawai leaf monkey	*P. potenziani*
Snub-nosed langurs	All *Pygathrix* (=*Rhinopithecus*)
African Cercopithecines	
Tana River mangabey	Cercocebus galeritus galeritus
Diana monkey	Cercopithecus diana
Drill	Papio (=*Mandrillus*) *leucophaeus*
Mandrill	*P.* (=*Mandrillus*) *sphinx*
African Colobines	
Zanzibar red colobus	*Colobus pennantii kirki*
Tana River red colobus	*C. rufomitratus*
New World monkeys	
Cebids	
Mantled howler	*Alouatta paliata* (=*villosa*)
Black-handed spider	*Ateles geoffroyi frontatus*
Black-handed spider	*A. geoffroyi panamensis*
Woolly spider	*Brachyteles arachnoides*
Uakaris	All species, *Cacajao* species
White-nosed saki	*Chiropotes albinasus*
Yellow-tailed woolly	*Lagothrix flavicauda*
Red-backed squirrel	*Saimiri oerstedii*
Callimiconids	
Goeldi's, Callimico	*Callimico goeldii*
Callitrichids	
White-eared marmoset	*Callithrix jacchus aurita*
Buff-headed marmoset	*C. jacchus flaviceps*
Golden lion tamarin	*Leontopithecus* (=*Leontideus*) spp.
Pied tamarin	*Saguinus bicolor*
White footed tamarin, silvery-brown bare-faced tamarin	*S. leucopus*
Cotton-top tamarin	*S. oedipus* (including *S.o.geoffroy*)
Tarsiers	None listed
Prosimians	
Daubentoniidae	
Aye-aye	*Daubentonia madagascariensis*
Indriidae	
Avahi	All species, *Avahi* spp.
Indri	All species, *Indri* spp.
Sifaka	All species, *Propithecus* spp.
Lemuridae	
Gentle lemur	All species, *Hapilemur* spp.
True lemur	All species, *Lemur* spp.
Megaladapidae	
Sportive lemur	*Lepilemur* spp.
Cheirogaleidae	
Dwarf lemur	All species, *Cheirogaleus* spp.
Hairy-eared dwarf lemur	*Allocebus trichotis*
Mouse lemur	All species, *Microcebus* spp.
Fork-marked mouse lemur	*Phaner* spp. [*P. furcifer*]

[a]All other nonhuman primates are listed in CITES Appendix II.

[b]All prosimian forms listed are Malagasian; of the Malagasy prosimians, only *Varecia* spp. and *Mirza coquereli* are absent from the list.

serves and regions of reputed abundance provide overestimates of density and must not be accepted uncritically as the basis for population estimates.

Survey and census data obtained or reported by individuals, groups, or institutions with special interests, biases, or investments in the outcomes of the population estimates are suspect. A willingness to support objective surveys is a strong indicator of good faith. If, for example, primate exporters claim that a species is abundant and that they should be allowed to continue capturing and exporting that species, and they resist efforts by objective individuals to conduct population surveys, there would be grounds for suspicion regarding the status of the populations in question and the credibility of the exporter. On the other hand, willingness of exporters to support objective surveys by qualified primatologists is a sign of good faith.

Protectionist groups have been critical for many years of the export of *M. fascicularis* from the Phillippines due to a lack of data on the wild populations. In 1987, primate exporters sought to initiate objective surveys, but instead of supporting the conduct of those surveys by protectionist groups that sought a total ban on the export of primates, they provided financial support to an indepedent agency qualified to conduct objective surveys. Protectionist groups opposed and blocked the studies. The population surveys still have not been conducted, the status of the populations is unknown, and the protectionists continue to use the *lack* of population data to support their campaign to ban primate exports. Likewise, proposals for objective surveys of wild chimpanzee populations were *opposed* by those who sought reclassification of all chimpanzees as endangered under the ESA, even though some of the individuals involved

TABLE III

NONHUMAN PRIMATES LISTED AS ENDANGERED UNDER U.S. ENDANGERED SPECIES ACT

Common name	Genus and species
Great apes	All species except chimpanzees in captivity
Lesser apes	All species
Gibbons	All species, *Hylobates* spp. (including *Nomascus*)
Siamang	*Symphalangus syndactylus*
Old World monkeys	
Asian Cercopithecines	
Macaque, lion-tailed	*Macaca silenus*
Asian Colobines	
Monkey, proboscus	*Nasalis larvatus*
Langur, capped	*Presbytis pileata*
Langur, Douc	*Pygathrix nemaeus*
Langur, entellus	*Presbytis entellus*
Langur, Francois'	*Presbytis francoisi*
Langur, golden	*Presbytis geei*
Langur, Pagi [sic] Island	*Nasalis (Simias) concolor*
Monkey, snub-nosed	All species, *Rhinopithecus* (=*Pygathrix*) spp.
African Ceropithecines	
Drill	*Papio leukophaeus*
Mandrill	*Papio sphinx*
Mangabey, Tana River	*Cercocebus galeritus*
Mangabey, white-collared	*Cercocebus torquatus*
Monkey, Diana	*Cercopithecus diana*
Monkey, L'hoest's	*Cercopithecus lhoesti*
Monkey, red-bellied	*Cercopithecus erythrogaster*
Monkey, red-eared nose-spotted	*Cercopithecus erythrotus*
African Colobines	
Monkey, black colobus	*Colobus satanus*
Monkey, Preuss' red colobus	*Colobus badius preussi*
Monkey, Tana River red colobus	*Colobus rufomitratus (badius) rufomitratus*
Monkey, Zanzibar red colobus	*Colobus kirki*
New World monkeys	
Cebids	
Monkey, howler	*Alouatta palliatta* (=*villosa*)
Monkey, red-backed squirrel	*Saimiri oerstedii*
Monkey, spider	*Ateles geoffroyi frontatus*
Monkey, spider	*Ateles geoffroyi panamensis*
Monkey, woolly spider	*Brachyteles arachnoides*
Monkey, yellow-tailed woolly	*Lagothrix flavicauda*
Saki, southern beared [sic]	*Chiropotes satanus satanus*
Saki, white-nosed	*Chiropotes albinasus*
Uakari, all species	*Cacajao* spp. (all species)
Callimiconids	
Marmoset [sic], Goeldi's	*Callimico goeldii*
Callitrichids	
Marmoset, buff-headed	*Callithrix flaviceps*
Marmoset, buffy tufted ear	*Callithrix jacchus aurita*
Marmoset [sic], cotton-top	*Saguinus oedipus*
Tamarin, golden rumped (=golden-headed tamarin; =golden-lion marmoset [sic])	*Leontopithecus* (=*Leontideus*) spp., all species
Tamarin, pied	*Saguinus bicolor*
Tarsiers	None listed
Prosimians	
Daubentoniidae	
Aye-aye	*Daubentonia madagascariensis*
Indriidae	
Avahi	All species, *Avahi* (=*Lichanotus*) *laniger* (entire genus)
Indri	All species, *Indri indri* (=entire genus)
Sifakas	All species, *Propithecus* spp. (all species)
Lemuridae	
Lemurs	*Lemuridae (incl. Cheirogaleidae, Lepilemuridae); all members of genera: Lemur, Phaner, Hapalemur, Lepilemur, Microcebus, Allocebus, Cheirogaleus, Varecia*
Megaladipidae: see Lemuridae	
Cheirogaleidae: see Lemuridae	

previously and subsequently advocated chimpanzee population surveys as a high priority activity. In both cases, the activists apparently believed that objective information would weaken their arguments.

Conservation and protection campaigns based on misinformation and deliberate understatements of primate populations damage the credibility of the entire conservation effort and impede the development and implementation of practical conservation plans. The temptation to engage in campaign rhetoric and debate must be resisted in favor of the relatively difficult task and obligation to objectively obtain and act on accurate evidence. The verity of the evidence depends on the methods used.

B. Population Monitoring Techniques

Several good sources of information on survey methods for monitoring primate (and other) populations are available (e.g., Burnham *et al.*, 1980; National Research Council, 1981; Southwick and Cadigan, 1972; Brockelman and Ali, 1987), and discussions of specific details of survey methodology are also available (Johns, 1985; Skorupa, 1987).

The value of population studies extends beyond the determination of conservation status. As Brockelman and Ali (1987) have pointed out, data on range, density, group structure, and abundance are essential to the understanding of systematics,

evolution, ecology, social behavior, epizootics, and zoogeography. Not surprisingly, detailed field studies from a variety of disciplines are major contributors to the understanding of primate conservation needs. Population studies conducted as part of a scientific program can usually provide more detail and background than surveys undertaken exclusively for the promotion of conservation. Careful surveys can provide an excellent bridge between multidisciplinary field research programs and the development of conservation action plans.

Brockelman and Ali (1987) have identified and discussed three types of population studies: (1) distributional surveys; (2) population sampling; and (3) intensive study of specific areas. They divide population sampling into two techniques: (1) strip transect and quadrat "sweep" surveys (Marsh and Wilson, 1981); and (2) line transect surveys (Southwick and Cadigan, 1972). A similar set of methods is outlined by MacKinnon (1986), who describes the following techniques used to estimate population densities: (1) intensive study; (2) sweep surveys; and (3) line transect surveys.

Population estimates are based on the following data: (1) determination of the average group size in various locations and habitats; (2) determination of the average number of groups per unit of land area (usually in hectares or square kilometers); and (3) determination of the total area of habitat remaining. Obviously, the precision of the final estimate depends entirely on the quality of the data used to calculate that estimate.

Group size estimates are based on actual counts of animals during sampling surveys. These surveys are usually conducted by walking along a predetermined line through the forest, stopping at regular intervals to visually scan and listen for vocalizations and sounds of movement. Brief surveys are usually conducted using existing trails or even roads, whereas longer-term studies usually use a grid matrix of trails cut at specific intervals and allowing all locations within the intensive study area to be surveyed. In intensive study areas, the sites of sleeping trees are often known, and groups can be comprehensively censused each morning and evening. Specific groups are often followed throughout the day as well and can sometimes be observed, counted, photographed, and/or videotaped as they cross clearings, roads, trails, or move in the forest canopy.

Detectability can vary greatly across species, habitats, and groups. Variation in the skill levels of surveyors and in the methods used by them can result in the differential detection of primates and apparent differences in average group sizes. Independent simultaneous counts by different observers and repeated sampling across time can serve as good checks on reliability, but the strongest evidence regarding group size comes from intensive long-term studies in which groups are habituated to the surveyor(s) but are not provisioned. Provisioning can result in attracting additional animals into a study area and can eliminate the possibility of obtaining unbiased information on group size and composition, feeding ecology, behavior, and activity budgets. The credibility of the results of many long-term primate projects has been permanently tarnished by provisioning. Brief surveys by experienced field primatologists can yield useful estimates of group size, but it is seldom possible to detect all members of each group during every encounter. Consequently, brief surveys are likely to underestimate group size relative to longer-term studies that often permit comprehensive censusing and identification of all individual members of each group studied, along with age and gender class and other demographic information.

Group density data, that is, the number of groups per unit of land area, are less subject to error than are estimates of average group size. This is simply because detection of a group is easier to do reliably than is the accurate counting of all members of the group once the group is detected. If one has a large body of data on group sizes, surveys intended just to document the number of groups within a specific area are relatively easy to conduct. Even so, coordinated simultaneous surveys by several individuals can reduce the resampling of groups and other sources of error.

The sweep and quadrat survey methods are intended to be more comprehensive than line transect techniques. Range mapping, more commonly done only during distributional studies or intensive studies, actually provides *census* data for specific groups and areas rather than just survey samples. Range mapping requires location of sleeping trees and progression sites to obtain absolute counts as well as documentation of feeding and resting sites throughout the day. Range maps for all groups within a confined area provide the strongest basis for density estimates and habitat use.

Brockelman and Ali (1987) recommend that intensive studies of at least a 1-year duration should be conducted before undertaking distributional surveys or population sampling studies because familiarity with habitat types, troop structure, typical densities, vocalizations, activity patterns, reactions to humans, and other factors can influence the outcomes of survey and sampling studies. Although this advice may be sound for those who lack experience with wildlife, forests, and survey methods, much of value can be learned when experienced professionals conduct repeated short-term distributional surveys and population sampling studies at many sites using standardized methods.

Surveys can be most effective when they are conducted by coordinated teams and when brief surveys at many sites are accompanied by intensive longer-term studies at a few critical sites. While individual students and lay naturalists can make useful contributions to primate conservation, experienced and professional multidisciplinary teams will be increasingly essential if primate conservation problems are to be addressed with competent planning and effective action. The participation of students and inexperienced field personnel in survey teams under the direct supervision of experienced field primatologists provides more survey coverage and reliability checks than could be accomplished by the professionals alone, while offering an efficient means of training personnel in survey methods.

V. METHODS OF CONSERVATION

Some methods of conservation discussed here focus on the action to be taken in the countries of origin; other conservation actions can be done in countries where primates are confined for research or exhibition. Activities that promote conservation include (1) reduced removal from wild populations by increasing captive propagation in countries of origin and countries of use; (2) reduction in the number of primates used up in terminal studies by coordinating compatible research uses and sharing biological material; (3) reduction in the total number of primates used in research by sequential involvement of each individual in research and reproduction; (4) reduction in the total number of primates used by centralization of multiple-use programs; (5) reintroduction or other wildlife management when conditions permit; (6) increased funding of projects that include studies of wild populations; and, most of all, (7) concerted efforts to promote population and habitat conservation in areas with natural primate populations.

A. Breeding Programs

Captive breeding programs contribute to primate conservation in several ways: (1) to serve as an alternative to wild capture for testing and biomedical and behavioral research; (2) to serve as an alternative to wild capture for exhibition in the service of conservation education in zoological gardens; (3) to serve as a means of perpetuating endangered species, especially for eventual reintroduction when possible; (4) to serve as centers for the comparative study of reproductive biology that supplies basic knowledge that can later be applied in the service of conservation; (5) to serve as models for epidemiological and health studies that may ultimately benefit wild nonhuman primates (e.g., by preventing the spread of infectious diseases in countries of origin); and (6) to develop husbandry and treatment procedures that improve survival rates of captured and purpose-bred primates.

Captive breeding programs for zoological exhibition and biomedical use are generally, but not always, kept separate. Among other reasons, this is because zoos usually emphasize captive propagation programs for rare and endangered species, whereas biomedical programs usually emphasize the most commonly studied (nonendangered) laboratory primates.

Zoo programs are coordinated through the Captive Breeding Specialist Group (CBSG), Species Survival Commission (SSC), International Union for the Conservation of Nature and Natural Resources (IUCN), and the Species Survival Program (SSP) of the American Association of Zoological Parks and Aquariums (AAZPA). The Species Survival Program coordinates specific Species Survival Plans (SSPs) for many primate species. Each species for which a SSP has been developed is managed by a species coordinator and a management committee. Representatives from participating institutions elect the members of the management committees and scientific advisors. The AAZPA currently sponsors SSPs for the primate species listed in Table IV.

TABLE IV

NONHUMAN PRIMATES FOR WHICH SPECIES SURVIVAL PLANS HAVE BEEN PREPARED THROUGH THE AMERICAN ASSOCIATION OF ZOOLOGICAL PARKS AND AQUARIUMS

Common name	Genus and species
Great apes	
Western lowland gorilla	*Gorilla gorilla gorilla*
Chimpanzee	*Pan troglodytes*
Bonobo ("pygmy chimpanzee")	*P. paniscus*
Orangutan	*Pongo pygmaeus*
Old World monkeys	
Lion-tailed macaque	*Macaca silenus*
Drill	*Mandrillus leucophaeus*
New World monkeys	
Golden lion tamarin	*Leontopithecus rosalia*
Prosimians	
Black lemur	*Lemur macaco*
Ruffed lemur	*Varecia variegata*

Biomedical breeding programs in the United States are primarily funded through the National Center for Research Resources (NCRR) of the National Institutes of Health (NIH). Most of these programs are operated by university research centers or by private industry under contract with NIH. Some breeding projects are supported through the U.S. Agency for International Development (AID) and the Pan American Health Organization (PAHO). Two of the major breeding programs sponsored by the NIH/NCRR are the Specific Pathogen Free (SPF) Monkey Breeding and Research Program (rhesus macaques) and the Chimpanzee Breeding and Research Program. NIH/NCRR also supports the National Primate Research Centers Program, and each of the seven regional primate research centers in the United States has breeding programs. The major breeding colony for pig-tailed macaques (*M. nemestrina*) at the Medical Lake breeding facility of the Regional Primate Research Center at the University of Washington is especially notable. The colony was was set up with specific goals for conservation as well as biomedical supply (Smith, 1975).

An island colony of rhesus monkeys was established in Puerto Rico in the 1940s. The island colony, Cayo Santiago, was established primarily for studies of social behavior but has become an important producer of rhesus monkeys. Other islands with introduced primate populations have become important sources of primates (St. Kitts, Barbados, and Mauritius).

In addition to breeding in countries of use, some breeding programs have been developed in countries of origin. The most notable of these are (1) the major program developed by SI-CONBREC in the Philippines for supply of cynomolgus macaques (*M. fascicularis*); (2) the Pulau Tinjil and Pulau Deli island breeding programs in Indonesia developed by Chuck Darsono (U.S. and Japanese support) for the supply of cynomolgus macaques; and (3) the breeding and cropping program

of the Center for Reproduction and Conservation of Nonhuman Primates at Iquitos, Peru.

B. Coordinated Use by Research Teams

Reduced numbers of wild-caught or purpose-bred primates could be achieved by increased coordination of research and testing uses. Major steps have already been made in that direction by increases in multidisciplinary program projects in which investigators from various fields collaborate on research involving a single set of research animals. An example of such a program was the "Prematurity in Primates" program project coordinated by G.P. Sackett of the Regional Primate Research Center at the University of Washington. A set of pig-tailed macaques from the RPRC breeding colony was identified from computerized records as being at very high risk or very low risk for poor pregnancy outcomes such as spontaneous abortion, stillbirth, low birth weight, or neonatal mortality. A team of scientists and clinicians, including pediatricians, neonatologists, endocrinologists, geneticists, physiologists, veterinarians, comparative psychologists, physical anthropologists, and neuroscientists, studied the same set of animals. Data from each discipline amplified the results of the others.

Another outstanding example of teamwork is described in "Behavior and Pathology of Aging in Rhesus Monkeys" (Davis and Leathers, 1985). Forty investigators from a remarkable array of disciplines and institutions all studied the same set of 15 rhesus monkeys (three of each of five age groups ranging from 4 to 31 years of age) in a highly coordinated manner. They related cross-sectional and longitudinal behavioral measures to age-graded pathology data on basic pathology, the nervous system, the endocrine system, the cardiovascular system, and the musculoskeletal system. The animals involved in the study had participated in hundreds of projects between 1952 and 1981. In the end, the contribution made by the study of 15 monkeys was greater than could have been achieved using thousands of monkeys in projects that were not coordinated with each other.

Unfortunately, regulations that limit the number of studies in which an animal can be involved have virtually eliminated the possibility of long-term sequential use studies. The effect of such regulations is a reduction in the quality of scientific information obtained, a dramatic increase in the cost of research, an extraordinary waste of animals, increased pressure on breeding colonies to produce more primates, and an increased demand for importation of primates. Blanket limitations on the number of studies a primate may be involved in can be very damaging with regard to conservation. Carefully coordinated multiple-use research programs can provide greatly refined scientific information while reducing the total number of primates required.

C. Information Exchange and Duplication

Critics of biomedical and behavioral research involving primates claim that much research is duplicative. Various programs have been proposed to assure that full literature reviews have been conducted before research protocols are approved; however, an excellent bibliographic service has been available for many years for those whose work involves nonhuman primates. The Primate Information Center at the Regional Primate Research Center at the University of Washington provides bibliographic services on the international scientific literature regarding research with nonhuman primates. *Current Primate References* is published monthly and provides thousands of new references each year. Custom bibliographies can be provided very rapidly by computerized searches of references from the past 25 years and beyond. Charges of duplication of effort due to inadequate access to the relevant scientific literature regarding primates are usually baseless, and the development of additional bibliographic resources is duplicative of the well-established and effective Primate Information Center. *Current Primate References* is also an excellent source of information on field research and serves an important role as a source of addresses for scientists whose work is relevant to conservation. The "International Directory of Primatology" (Jacobsen, 1992) from the Wisconsin Regional Primate Research Center provides detailed listings of primate information resources and other items of interest regarding primates and primate conservation.

The Primate Information Center also aids the coordination of primate use through its Primate Supply Information Clearinghouse (PSIC). The PSIC promotes communication to facilitate exchanges of primates and their tissues across institutions (including both research institutions and zoological parks). In addition, the PSIC maintains a database of programs, sources, services, equipment, and available or wanted animals. The information provided by the PSIC clearly reduces the total number of primates that must be taken from the wild or be purpose bred for research use.

Professional societies and journals also promote the exchange of information relevant to primate conservation. The International Primatological Society meets every second year. Affiliated societies, such as the American Society of Primatologists, the Primate Society of Great Britain, the Francophone Society of Primatology, the Latin American Primatological Society, and the Primate Society of Mexico, meet more often. The Association of Primate Veterinarians usually meets in association with the American Association of Laboratory Animal Science (AALAS). Several scientific journals are devoted specifically to primates, notably, *Primates* (Japan), *Folia Primatologica* (Switzerland), *Journal of Medical Primatology* (United States/Denmark), *International Journal of Primatology* (United States), and *American Journal of Primatology* (United States).

D. Reintroduction

The ultimate goal of captive propagation programs for endangered primate species is that progeny may eventually be reintroduced to natural habitat and may become self-sustaining

in that context. For a variety of reasons, reintroduction is seldom possible or practical, and unless the circumstances are just right, the consequences of reintroduction can be disastrous for the wild population.

Attempts to reintroduce chimpanzees in Africa and orangutans in Indonesia and Malaysia have been marginally successful, at best. Probably the most successful reintroduction demonstration for conservation purposes has been the reintroduction of the golden lion tamarin (*Leontopithecus rosalia*) to the Pocos d'Antos forest in Brazil (Kleiman *et al.*, 1986). The total wild population had fallen to less than 200. Advances in the understanding of social organization, reproductive biology and behavior, genetic management, and veterinary care led to a highly successful breeding program in zoological gardens. With the approval of the AAZPA/SSP Management Committee for this species, primatologists Devra Kleiman and Benjamin Beck at the National Zoological Park coordinated a reintroduction program. Tamarins bred in United States zoos were moved to the Centro de Primatologia to Rio de Janeiro. The reintroduction program included a field research and monitoring program and a highly successful public conservation education component. Perhaps the greatest achievement of the golden lion tamarin program was its value as a model for understanding the obstacles to successful reintroduction and the costs and benefits of including reintroduction as a part of the captive propagation effort.

Reintroduction is often not feasible because no suitable habitat is available. After all, the primary threat to wild primate populations is habitat loss. If, however, populations have been removed from an appropriate habitat by hunting or trapping, the prospects of successful reintroduction can be very strong. When reintroduction is possible due to the presence of a good habitat, it may not be feasible for other reasons. Care must be exercised not to introduce pathogens that have been picked up in captivity into the wild, either to conspecifics or across species. Adequate protection from undue conflict with humans in the area of introduction must be assured. The reintroduction of animals that will become agricultural pests or may present zoonotic or other hazards to human health is not a viable conservation strategy.

E. Translocation

The movement of groups or entire populations from one setting to another is an appropriate and well-established wildlife management technique and is becoming increasingly feasible and important for conservation of primate populations whose habitats have been destroyed or limited by human activities (cf. Strum and Southwick, 1986).

Strum and collegues translocated three groups of baboons from a ranch at Gilgil, Kenya, to the Laikipia Plateau about 120 miles away. These groups had been the subject of longitudinal behavioral research studies, and the decision to move them as intact groups was made in an attempt to preserve the groups for continued research and to evaluate the potential of using translocation as a conservation strategy for primates. A total of 131 baboons were trapped and moved without any deaths and only one serious injury. Groups remained intact and adjusted to their new surroundings within 3 months. In two of the three groups no mortality followed release at the new site. Some losses occurred in the other group which was not temporarily provisioned as the others were. Within a few weeks after introduction, members of the introduced groups began to assimilate with the resident population. Normal patterns of reproduction were not disrupted by the move.

A subgroup of 20 rhesus monkeys in Aligarh District, India, was moved from a group of 130 individuals to a site 30 km away that had been previously occupied by rhesus monkeys (Southwick *et al.*, 1984). The habitat into which the monkeys were introduced was near a canal bank and was judged superior to the original habitat. They were originally provisioned as part of the reintroduction protocol, but were later moved across the canal where they were adopted by villagers who supplemented their diet and regarded them as a gift from the monkey god, Hanuman. The trapping, transport, and relocation did not influence reproductive success and no mortality was associated with the translocation project.

Based on these experiences and a review of the related literature, Strum and Southwick (1986) identified the factors that were most important to successful translocation. These included (1) composition and nature of the group; (2) characteristics of the release site; and (3) the specific procedures involved in capture, transport, release, and monitoring. Their recommendations were that (1) translocation should involve intact social units; (2) groups should not be moved into protected areas harboring healthy resident populations of the same species; (3) either declining or expanding populations are candidates for translocation; and (4) translocation potential should be evaluated using nonendangered primate species.

Translocation sometimes provides a viable alternative to other options such as protection *in situ* against insurmountable odds, letting the animals die, or taking them into captivity; however, the most difficult problem faced when contemplating translocation is the identification of a suitable site for the animals to be introduced. Local people are usually not receptive, with good reason, to the introduction of potential agricultural pests near their villages, gardens, or fields. They are much more receptive to the concept of moving monkeys away from their area. Moving animals outside the natural range of the species is problematic as well. Systematic zoologists usually oppose translocation because it can hopelessly obscure natural population patterns. The introduction of monkeys into areas where they have been absent can have devastating effects on local fauna and flora. Perhaps the best situation one can hope for is an adequately protected area with an abundance of appropriate habitat that was previously (and recently) occupied by the species to be introduced and where the distance is sufficiently remote from attractive crops but not too far from the site of origin of the group. Situations of this kind are most likely to be found in areas where

the species has been eliminated by overhunting, so the provision of adequate protection is likely to be especially important.

F. Conservation *in Situ*

By far the most important kind of conservation, and the goal to which all other methods should ultimately be directed, is the conservation and protection of natural primate populations in the ecosystems to which they are adapted.

VI. CONCLUSIONS

Primate conservation requires a team effort by scientists, clinicians, professionals, and local people in countries of primate origin. Primate users can benefit from becoming knowledgeable about the species with which they work. Detailed scientific information on each primate species in captivity and in the wild is ultimately in the best interests of conservation. As more information becomes available, and as that information is placed in a broad comparative context, decisions about appropriate species choice for specific studies will become increasingly refined. The interplay of information from laboratories and natural settings will increase the motivation and enhance the ability of scientists to engage in effective programs of primate conservation. Finally, we must emphasize the critical importance of careful management of primate populations and the habitats in which they live. This is a vital responsibility that requires local action aided by leadership from the scientific and political communities.

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CHAPTER 8

Genetics of Nonhuman Primates

John L. VandeBerg

I. INTRODUCTION

An understanding of genetics is fundamental to the use of nonhuman primates for biomedical research for several reasons. First, genetic differences are responsible for much of the basic physiological variability that exists among species and among individuals within species. Even variation in response to natural or experimentally controlled environmental factors is largely under genetic control. Any attempt to understand fully the basis of physiological differences among species or individuals must include an evaluation of those differences at the level of variation in genes or proteins encoded by the genes, and at the level of gene expression. Second, genetic management procedures are essential for the long-term success and efficiency of captive breeding programs, which have become important because of the severely reduced availability of wild-caught nonhuman primates. Third, the definition of genetic markers during recent decades provides new opportunities for powerful research strategies to answer fundamental questions in biomedical research. And fourth, a detailed understanding of genetic similarities and differences between humans and their closest relatives is essen-

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tial for the development and appropriate use of nonhuman primate models. One of the most exciting new uses of nonhuman primate models is the development and testing of gene therapy technologies, which undoubtedly will become commonplace in the 21st century.

Historically, genetics has been underrepresented in research with nonhuman primates (reviewed by VandeBerg, 1987). This neglect was in part a consequence of geneticists focusing their attention on humans or on model organisms that are more tractable for genetic research (e.g., those with short generation times) and in part a consequence of primatologists focusing their attention on complex biological characteristics far removed from the genes and their patterns of expression. However, the trend toward increasing genetic emphasis in biomedical research with nonhuman primates is readily apparent from the number of genetics-related publications indexed in MEDLARS files (VandeBerg, 1987). During the 20-year span from 1966 to 1985, the proportion of publications involving genetic information, among all publications involving nonhuman primates, rose from 41 to 83%. During the same period, the proportion of publications that focused primarily on genetics, among all publications involving nonhuman primates, increased sixfold from 3 to 18%. This increased level of genetic research has been stimulated by growing recognition of the fundamental role of genetics in determining physiological and behavioral characteristics, including those related to human disease; by the development of pedigreed breeding colonies that provide opportunities for genetic approaches in biomedical research; and by the development of powerful techniques for detecting and characterizing genetic markers at the levels of chromosomes, proteins, and DNA.

This chapter begins with an overview of the genetic relationships between nonhuman primates and humans. The overview is followed by discussion of the four types of genetic markers derived from the subdisciplines of cytogenetics, immunogenetics, biochemical genetics, and molecular genetics. These genetic markers provide the basis for almost all genetic management and genetic research conducted with nonhuman primates. After an explanation of some technical considerations regarding the use of genetic markers, some applications of genetic concepts to the management of captive breeding colonies are discussed. The chapter concludes with a discussion of strategies for developing animal models for biomedical research.

II. GENETIC RELATIONSHIPS OF NONHUMAN PRIMATE SPECIES AND MAN

A. Genetic Similarities

The scientific value of nonhuman primates for biomedical research derives from the many anatomical and physiological characteristics that they share with humans because of a close phylogenetic relationship and a short duration of evolutionary divergence. Because of these similarities, only nonhuman primates are suitable animal models for many human behavioral and physiological processes in health and disease. Many of the similarities among primate species (including humans) are obvious at the gross anatomical, physiological, and behavioral levels, but only in recent decades has it been possible to quantify the underlying genetic basis of those similarities.

1. Chromosomal Arrangements of Genes

Although the number of chromosomes differs among the primate species, modern chromosomal banding techniques have provided strong evidence that the karyotypes of many primate species have been derived during evolution by relatively few fission, fusion, translocation, and inversion events. For example, each of the 46 human chromosomes has a recognizable counterpart in the great apes except for chromosome 2, which exists in the great apes as two separate chromosomes, each homologous to one of the arms of human chromosome 2. Thus, the great apes have a diploid chromosome number of 48. Baboons and rhesus monkeys, on the other hand, have a diploid chromosome number of 42 [and identical karyotypes (Finaz *et al.*, 1978; Dutrillaux *et al.*, 1979)], and their chromosomal banding patterns also reveal almost complete correspondence with those of human chromosomes, with a few rearrangements (Dutrillaux *et al.*, 1978). The evolutionary events that gave rise to the various karyotypes have been deciphered in great detail for many primate species (reviewed by DeGrouchy, 1987). In contrast, examination of banded karyotypes reveals no, or at best only limited, homologies between chromosomes of humans and those of species in other mammalian orders, indicating that only nonhuman primates are highly similar to humans in the chromosomal arrangements of their genes.

In general, the extent of karyotypic similarity is a function of phylogenetic relatedness, so fewer chromosomal homologies exist between New World monkeys and humans than between Old World monkeys and humans (Dutrillaux, 1979). An exception to this generality, however, is the gibbons (family Hylobatidae), which belong to the superfamily Hominoidea along with humans and the great apes. The karyotypes of gibbon species have apparently undergone many alterations during a short evolutionary period of history so that homologies with human chromosomes can no longer be recognized (Dutrillaux, 1979).

It might be expected to follow from the karyotypic conservation of primate chromosomes and their banding patterns that the linear arrangement of genes along each chromosome would be conserved in parallel with the linear arrangement of chromosomal bands. Genes that are present on the same chromosome are said to be "syntenic," and comparative gene mapping studies have revealed that most syntenies have indeed been conserved during primate evolution (reviewed by Seuánez, 1987; see also Lalley *et al.*, 1989). Little is known yet about the actual order of genes on the chromosomes of any nonhuman primate species, but research in progress is ex-

pected to confirm the conservation of gene order among species with conserved karyotypes.

The functional significance of conserved karyotypes and syntenies was illustrated by the discovery that a chimpanzee that exhibited characteristics of Down's syndrome was trisomic for a small acrocentric chromosome (McClure *et al.*, 1969) with the characteristics of human chromosomes 21 (Benirschke *et al.*, 1974). Subsequently, chromosomal banding analyses have revealed that trisomy of orangutan chromosome 22 (the homolog of human chromosome 21) also is associated with the symptoms of Down's syndrome (Andrle *et al.*, 1979).

2. DNA Sequences

The most refined level at which genetic similarity can be measured is the DNA nucleotide base sequences of the nuclear genes. Similarity is generally greater in coding sequences, which are subject to natural selection acting on the characteristics of the proteins produced, than in noncoding sequences. Therefore, a maximal estimate of divergence can be obtained by comparing noncoding sequences. A typical comparison conducted by Li *et al.* (1987) for globin pseudogenes (nontranscribed genes) revealed that humans have 98.3% sequence identity with chimpanzees, 96.9% with orangutans, 92.7% with rhesus macaques, and 88.8% with owl monkeys. In comparison, rhesus and owl monkeys exhibit 86.7% sequence identity. These limited data (i.e., from a very small sample of DNA sequences) suggest that owl monkeys are about as distantly related to rhesus macaques as they are to humans.

3. Protein Structure

It follows from the conservation of nucleotide base sequences in primate DNA that the proteins encoded by the genes also must be highly conserved. Indeed, the amino acid sequences of proteins are even more highly conserved than nucleotide base sequences for three reasons. First, the genetic code contains synonyms (degeneracy) in that most amino acids are specified by more than one nucleotide triplet (codon). Therefore, some nucleotide base substitutions do not lead to amino acid substitutions. Second, much of the DNA exists as intergenic sequences, which are interspersed between the structural genes that encode the proteins. Some of that DNA is involved in the regulation of gene expression, but most of it has no known function and is not constrained from evolutionary change by the selective forces that reduce variability in coding sequences. Third, structural genes contain sequences, called "introns," which are transcribed but not translated, interspersed with the exons whose mRNA is translated into the amino acid sequences of the proteins. Much of the variation in structural gene sequences in the introns is not subjected to the same selective pressures.

Data support these theoretical predictions. For example, from results obtained by electrophoresis of 44 different proteins, it has been concluded that human and chimpanzee proteins share an average of more than 99% of their amino acid sequences (King and Wilson, 1975). Based on these results, the genetic distance between humans and chimpanzees is equivalent to that between sibling (i.e., morphologically indistinguishable) species of other taxa.

Because the amino acid sequences (and functional characteristics) of proteins of humans and chimpanzees are so remarkably similar, it has been concluded that the differences between these species are determined primarily by differences in gene expression patterns, which are controlled by regulatory genes (Bruce and Ayala, 1978). Apparently, small differences in the timing and extent of expression of the 50,000–100,000 structural genes estimated to exist in the haploid genome account for most of the phenotypic variation between humans and chimpanzees.

B. Unique Characteristics of Different Species

Despite the many genetically controlled similarities among primate species, genetic differences among the species are fundamentally important in choosing the appropriate species for specific biomedical research purposes. A few examples will illustrate the care required in choice of research subjects.

An obvious example is choice of species for research that requires infection with the human immunodeficiency virus (HIV), which causes acquired immunodeficiency syndrome (AIDS). Of all the primate (and nonprimate) species available for research purposes, only chimpanzees can be consistently and persistently infected with HIV, probably because they alone have a specific cellular receptor sufficiently similar to the human receptor that enables the virus to gain entry into T4 lymphocytes. However, unlike humans, infected chimpanzees do not develop the clinical symptoms associated with the acquired immunodeficiency syndrome.

A less obvious example involves the lipemic response to dietary cholesterol and fat. Several species of nonhuman primates are used frequently in research on genetic and dietary effects on concentrations of plasma lipoproteins and on development of atherosclerosis. Whereas baboons and rhesus macaques have identical banded karyotypes, they have dramatically different responses to dietary cholesterol and fat. Most baboons exhibit a modest lipemic response similar to that of most humans, and the resultant arterial lesions develop at a modest rate and resemble the early stage atherosclerotic lesions that are present in many humans. In contrast, most rhesus macaques are hyperresponsive to dietary fat and cholesterol and rapidly develop arterial lesions that resemble those in persons with extremely high plasma cholesterol levels. The choice of which species to use in research on lipoproteins and atherosclerosis clearly depends on what questions are being asked.

Another example where choice of species is critical is in research on malaria, which is caused by infection with *Plasmodium* species. Squirrel monkeys are generally used for such

research and for testing vaccines against *Plasmodium*, but not all squirrel monkeys are appropriate. For example, Guyanese squirrel monkeys (*Saimiri sciureus*) are susceptible, whereas Bolivian squirrel monkeys (*Saimiri boliviensis*) are not. Susceptibility is believed to be related to the presence of Duffy blood group antigens on the surface of red blood cells (Miller *et al.*, 1975; Nichols *et al.*, 1987). These antigens serve as receptors by which the *Plasmodium* merozoites gain entry into the cells.

Subspecies also represent different gene pools whose unique characteristics should be considered in selection of research subjects. For example, baboons are currently classified as a single species (*Papio hamadryas* Linnaeus 1758) on the basis of genetic and reproductive characteristics (Williams-Blangero *et al.*, 1990), but only red baboons (*Papio hamadryas papio* Desmarest 1820) are favorable models for photosensitive epilepsy (Meldrum and Wilkins, 1984).

Combining data on animals selected from genetically different populations can seriously bias experimental results. For example, combining data on a qualitative risk factor for disease from different subpopulations can hide an association between the risk factor and disease when one is present or create a spurious association that is not biologically relevant (Sturt, 1984). The genetic heterogeneity present among populations and subpopulations of animals should be addressed explicitly in experimental designs (Williams-Blangero *et al.*, 1990).

III. GENETIC MARKERS

A. Cytogenetic Markers

All genetic markers have two fundamental characteristics: (1) they are discrete variants in the structure of chromosomes, proteins, DNA, or any other characteristic of an organism; and (2) they exhibit Mendelian patterns of inheritance. Genetic markers are the tools that enable modern genetic research and management initiatives.

The discovery of the modern chromosomal staining technologies in the early 1970s made possible the identification of karyotypic variation at a much finer level of detail than previously had been possible. The techniques include C-, G-, Q-, R-, and T-banding as well as G-11 banding and Hoechst 33258 banding. Each of these techniques provides distinct patterns of light and dark bands along the chromosomes as a consequence of particular physicochemical characteristics at each site. The most commonly used of these techniques are C-banding and G-banding. Both of these techniques involve the use of Giemsa stain, which is a complex mixture of dyes of various degrees of oxidation.

The C-banding procedure stains regions that contain constitutive heterochromatin, which reflects large blocks of DNA that are transcriptionally inert on a permanent basis. C-bands are typically present at the centromeres, but may exist at other chromosomal locations. Polymorphisms are detected as the presence or absence of particular C-bands or as differences in size, and these variants exhibit typical patterns of Mendelian inheritance. An example of such a polymorphism exists on chromosome 14 of squirrel monkeys. It has been demonstrated that 19% of Guyanese squirrel monkey chromosomes 14 and 39% of Bolivian squirrel monkey chromosomes 14 exhibit a large C-band on the short arm; the other chromosomes 14 in these two types of squirrel monkeys did not exhibit a C-band at this site (Moore *et al.*, 1990). This C-band is so large that its presence more than doubles the size of the short arm of chromosome 14.

The G-banding procedure enhances the intensity of bands that exist as a consequence of the subchromosomal organization of DNA into chromomeres (reviewed by Comings, 1978). G-banding can be highly informative because several hundred bands can be detected in a karyotype, and under certain conditions as many as several thousand bands are visible. In the first examination of high-resolution chromosomes (800-band level) of rhesus monkeys, Small *et al.* (1985) detected three G-band polymorphisms in a sample size of only three individuals. This result suggests that high resolution G-banding is potentially a powerful technique for detecting cytogenetic markers in nonhuman primates.

Silver staining identifies nucleolar organizer regions (NORs), in which mRNA is transcribed. In some species, the NORs exhibit polymorphism in size; these polymorphisms presumably reflect a variation in the rate of mRNA synthesis by each NOR. For example, a cytogenetic analysis of NORs in 26 Peruvian squirrel monkeys revealed that 3 of the 52 chromosomes 2 had an unusually small NOR, whereas 2 of the chromosomes 2 had an unusually large NOR (Moore *et al.*, 1990). These NOR size variants are inherited as simple codominant traits.

A new cytogenetic strategy has been developed for determining which nonhuman primate chromosomes or chromosomal fragments are homologous with human chromosomes (Wienberg *et al.*, 1990). This strategy involves isolating many copies of a single human chromosome by flow sorting, labeling the DNA with biotin, hybridizing the labeled DNA to nonhuman primate chromosomes, and visualizing its location microscopically by the use of fluorescein-conjugated avidin. This technology will be useful for comparative gene mapping by determining the chromosomal locations of blocks of genes in nonhuman primates that have been well characterized in humans.

Although cytogenetic techniques can provide substantial information, they are expensive and time-consuming; it is not practical to apply them routinely in large-scale population studies. Their use is primarily to compare species and subspecies for the purposes of addressing evolutionary questions and implementing genetic management procedures, as discussed in Section IV.

B. Immunogenetic Markers

1. Red Blood Cell Markers

Discovery of the A–B–O blood group system in humans in the early 1900s led ultimately to the techniques for identifying many genetically controlled intraspecies differences in blood group antigens in humans (Race and Sanger, 1975) and experimental animals (Erskine and Socha, 1978). Allelic variants within a blood group system result not from variations in amino acid sequences of cell surface proteins, but rather from variation in the oligosaccharide components of glycoproteins and glycolipids associated with the cell surface. These variants are controlled by genetic variation in the enzymes responsible for attaching the sugar moieties. Thus, the A blood group antigen arises from the presence of an enzyme that adds a galactosamine to the basic A–B–O antigen molecule, whereas the B blood group arises from the presence of an allelic enzyme that adds a galactose (Watkins, 1980).

The genes that control the A–B–O system of humans are also present and functional in many nonhuman primates, and blood from apes can be typed for A–B–O in the same manner as human blood. Other primates do not have the A or B antigens on their erythrocytes, but these antigens are present on other cell types and in secretions, as in humans, and isoagglutinins are present in their plasma. Therefore, A–B–O genotypes can be determined in these species from buccal mucosa epithelial cell smears (Nehlsen-Cannarella and Bohn, 1987) or by indirect techniques that use saliva or serum (Wiener and Moor-Jankowski, 1969).

Nonhuman primate blood groups defined with reagents developed for typing human blood (such as A–B–O) are called "human-type blood groups" and are presumed to be the evolutionary homologs of their human counterparts. Most reagents for human blood group typing detect homologous antigens on blood cells of most nonhuman primate species (Eckstein *et al.*, 1988), but these human-type blood groups usually consist of a single antigenic type in nonhuman primates rather than two or more allelic forms. However, some polymorphisms have been detected in nonhuman primates, primarily in the apes, in human-type blood groups other than A–B–O (reviewed by Socha, 1989).

In order to develop typing reagents that detect polymorphic blood groups in nonhuman primates, it was necessary to use red blood cells to cross-immunize individuals within species (alloimmunization). Occasionally, such reagents were produced by immunizing another species with primate red blood cells. Blood groups identified with antisera produced directly against nonhuman primate red blood cells are called "simian-type blood groups." Simian-type blood groups have been developed extensively in rhesus monkeys, for which over two dozen operationally monospecific blood typing reagents detect alleles at 16 different gene loci (Stone *et al.*, 1987a; Frederick *et al.*, 1990).

Most of the blood group polymorphisms for nonhuman primates are "open" systems. Thus, if only a single antigen is detected on the red cells of an individual, it is not known if the individual is homozygous for the gene specifying that antigen or is heterozygous for that gene and for a "null" allele, which specifies no antigen (or specifies an antigen for which no blood group reagent exists). Although this problem limits the utility of blood groups, the combined genetic power of many open blood systems is nonetheless substantial. In addition to their intrinsic interest, blood group data have made important contributions to research in population genetics (e.g., see McMillan and Duggleby, 1981), to applications in genetic management (see Smith, 1982, 1985), and to the maintenance of accurate pedigrees (see Sullivan *et al.*, 1977; Curie-Cohen *et al.*, 1983a).

Despite the contributions of blood group systems to nonhuman primate research and management objectives, the important of blood groups is likely to diminish in the future. The blood group reagents are laborious to produce, and the scientific community has depended on the generosity of two principal laboratories for the production of reagents (see reviews by Stone *et al.*, 1981; Socha *et al.*, 1984). The powerful protein electrophoresis technologies developed in the 1960s and recombinant DNA technologies developed in the 1980s have already revealed polymorphisms in thousands of human gene loci and in scores of gene loci in each of many nonhuman primate species. These procedures are economical, can identify polymorphisms at an almost unlimited number of gene loci, and permit unambiguous inference of genotype from phenotype.

2. White Blood Cell Markers

The early work of Balner and associates (reviewed by Balner, 1977) established unequivocally that nonhuman primates have a cluster of genes that is amazingly similar in structure and arrangement to the human major histocompatibility complex (MHC). Balner's conclusions, based on results from rhesus monkeys and to a lesser extent from chimpanzees, have been supported by more recent studies of these and other Old World primate species (Jonker *et al.*, 1982; Heise *et al.*, 1987).

Most genes within the MHC specify cell surface glycoproteins that control the recognition and response of the immune system. Class I MHC glycoproteins, which are involved in allograft rejections and in the destruction of virus-infected cells, have been studied in detail with antisera that detect extensive polymorphism via cytotoxic effects on lymphocytes. These antisera are derived primarily from multiparous females, which have been naturally immunized against blood of their fetuses, or by alloimmunization or skin grafting. Class II MHC glycoproteins, which are required for presenting processed antigens to helper and suppressor T cells, exhibit somewhat less polymorphism than Class I glycoproteins. Class II molecules are the major stimulating antigens in allogeneic proliferative responses and can be characterized genetically by serological techniques, which detect broad antigen specificities, or by

mixed lymphocyte cultures, which quantify the ability of lymphocytes to proliferate in response to the presence of lymphocytes from another individual.

Although these classical approaches in MHC research are valuable and necessary for research on immune function, they are laborious and expensive procedures for genetic characterization by comparison with modern molecular technologies. It is now more practical to identify genetic variation in MHC markers by molecular genetic analysis at the DNA level, as discussed in Section III. Molecular methods of studying genetic variation in MHC genes of nonhuman primates have been used extensively to enhance our understanding of the evolution of the MHC (Lawlor *et al.*, 1988; Mayer *et al.*, 1988; Bontrop *et al.*, 1989; see review by Lawlor *et al.*, 1990).

C. Biochemical Genetic Markers

Biochemical genetic markers are genetic variants of enzymes and other proteins, and are most commonly detected by electrophoretic or isoelectric focusing techniques. These methods can discriminate allelic proteins with different surface charges due to amino acid substitution. Other biochemical characteristics in which allelic variation exists are molecular weight (also detected electrophoretically) and, for enzymes, the level of activity. Although proteins from any tissue can be subjected to these analyses, biochemical genetic research with nonhuman primates generally involves red blood cells, serum or plasma, and occasionally white blood cells, simply because blood can be readily obtained without harm to the animal.

Biochemical genetic markers are extensively used for several reasons. First, they do not require the development of specialized reagents such as those required for serological testing of white blood cell markers or for characterizing simian-type blood groups. Electrophoretic techniques developed in research with human blood are usually adaptable to blood of nonhuman primates with little if any modification. Second, electrophoretic analysis reveals the allelic gene products as distinct bands on gels, whereas red and white blood cell typing enables only the inference of allelic gene products. Third, the number of proteins that can be subjected to electrophoretic analysis is limited only by the availability of histochemical and immunochemical techniques to identify each specific protein of interest. Commercially available antisera against specific human proteins are usually specific for the same nonhuman primate proteins. Several hundred gene products that are present in blood components can be examined for polymorphism by electrophoretic techniques, which typically reveal polymorphism in about 25% of the proteins surveyed in any particular species. Fourth, electrophoretic analysis of proteins can be performed with minute quantities (a few microliters) of cell extracts or serum, so many markers can be typed from a limited sample volume. Fifth, because biochemical genetic techniques detect alterations in protein structure, some biochemical genetic markers reflect differences related to altered physiological conditions. Because of these advantages, biochemical genetic markers have largely superseded immunogenetic markers for genetic research and management objectives. A review of some uses of biochemical genetic markers in research with nonhuman primates was provided by Meera Khan (1987).

Limitations of biochemical genetic markers are that (1) only proteins that are present in blood are accessible to biochemical genetic analysis, unless other tissues are collected; and (2) a relatively large volume of blood is required to obtain enough white blood cells for extensive biochemical genetic analysis of mitochondrial enzymes, which comprise an important class of polymorphic markers.

D. Molecular Genetic Markers

1. Classes of Genetic Markers

The rapid development of several classes of molecular genetic (i.e., DNA) markers has greatly increased the power of genetic analysis and genetic management of nonhuman primates and has provided new opportunities for developing nonhuman primate models of human diseases.

Nuclear DNA markers can be classified into three major groups: (1) point mutations which consist of single nucleotide base substitutions, (2) insertions or deletions of single or multiple bases within a single copy of a base sequence, and (3) differential numbers of copies of tandem repetitive sequences.

This third class can be further subdivided into two subgroups based on the type of repeated sequence involved: (1) the variable number of tandem repeats (VNTRs) of short oligonucleotide sequences (11 to 60 base pairs), and (2) the variable number of dinucleotide repeats (VNDRs), e.g., cytosine–adenine or CA repeats.

2. Southern Blotting Technique for Identifying Variation in Single-Copy Sequences

Some point mutations, insertions, and deletions in the nucleotide base sequences of single-copy DNA can be detected as polymorphic markers called restriction fragment length polymorphisms (RFLPs) (Botstein *et al.*, 1980). These markers can be identified in DNA isolated from any tissue or from cultured cells, although white blood cells are generally used as the most convenient source of DNA. The DNA is digested with any one of a large number of restriction enzymes (endonucleases) which cut the DNA only at sites where a specific recognition sequence of nucleotide bases exists. The most commonly used restriction enzymes recognize sequences of four or six bases. Any alteration of the precise sequence, such as point mutation, will cause failure of recognition and, hence, failure to cleave the DNA. After the DNA is digested by a particular restriction enzyme, it is subjected to an electrophoretic procedure that separates DNA fragments according to their size in agarose gels. The DNA then

is transferred to and immobilized on a nitrocellulose or nylon filter. The transfer of DNA to the filter and the subsequent detection of the location of specific sequences are known as "Southern blotting." In order to visualize the DNA from the specific gene(s) of interest, a radioactive (or otherwise labeled) DNA sequence (i.e., a probe) that is complementary to the sequence of interest is allowed to bind to the immobilized DNA, and its location is detected by autoradiography (Southern, 1975) or by nonisotopic detection methods such as biotin–avidin staining (Dykes, 1988). The probes are constructed as complementary sequences of known functional genes or they can be anonymous probes which are complementary to sequences that are not derived from a known gene. If a particular recognition sequence is present in the DNA of one individual but not another, the fragments derived from the two DNA samples after digestion with the appropriate restriction enzyme will be different sizes and thus will migrate at different rates in the agarose gel. Consequently, they will end up at different places on the gel and on the filter. Alternatively, if the number of bases between restriction sites differs between individuals due to an insertion or deletion, the fragments also will be different sizes.

Most RFLPs of single-copy sequences, like biochemical genetic markers, are inherited as simple codominant traits in closed genetic systems. They can be detected in or near almost every gene for which DNA probes exist, whether or not that gene is actually transcribed. Therefore, this technology enables the detection of polymorphism in almost any gene targeted for a particular research program, and eventually the technology will enable thousands of genes to be used as markers. This is an important advantage of RFLPs by comparison with biochemical markers. By 1989, RFLPs had already been identified at nearly 1900 sites in the human genome, including about 400 sites in or near defined genes and about 1500 sites at which a gene had not yet been defined (Kidd *et al.*, 1989).

A limitation of RFLPs is that they are generally more expensive to develop and to use than biochemical genetic markers on a per marker basis. Also, most RFLPs consist of only two allelic forms, which represent either presence or absence of a particular restriction site. In contrast, biochemical markers frequently exhibit 3–5 allelic forms (and occasionally up to 10 or more) that can be resolved on a single gel (see VandeBerg, 1983, 1992, for numbers of alleles detected for biochemical genetic markers of rhesus macaques and baboons). Although multiple alleles at a particular locus can often be identified by the use of several restriction enzymes or probes, this strategy is economically practical only when it is important to develop and use a particular locus as a highly polymorphic marker.

Another disadvantage of RFLPs is that most of the DNA variants exist in noncoding DNA sequences. Indeed, it is generally accepted that only 1–2% of the primate genome codes for proteins (Lewin, 1990), and the noncoding regions are more variable than the coding regions because they are less constrained by selective forces. Therefore, the potential of RFLPs for physiological relevance is more limited than that of biochemical genetic markers, which involve structural alteration of proteins. In some instances, those structural alterations have functional effects.

3. Southern Blotting Technique for Identifying VNTR Polymorphisms

The existence of (VNTRs) provides a means of detecting a substantial number of alleles at some loci by the use of a single restriction enzyme and probe (Nakamura *et al.*, 1987, 1988). Most VNTRs are detected with anonymous probes, but occasionally they exist adjacent to a structural gene and can be detected with a probe that is complementary to sequences in that gene. In either case, if a probe detects sequences that are located adjacent to sequences that are repeated a variable number of times in different individuals, then it is possible to detect the VNTR by Southern blotting through the use of a restriction enyzme to cleave DNA into small fragments (e.g., <15 kb) appropriate for electrophoretic separation. The size of the DNA fragments will be a function of the number of tandem repeats in that particular DNA sequence. Because the number of tandem repeats is often highly variable in a population, many alleles at a particular locus or an anonymous site in the genome can be detected. Although the alleles rarely reflect the existence of variation in the coding sequences of a functional gene, they do enable the detection of a high frequency of heterozygotes required for many genetic purposes. By 1988, about 100 sites with VNTRs had been identified in the human genome (Nakamura *et al.*, 1987, 1988).

4. DNA Fingerprinting

DNA fingerprinting uses the same procedures that are used for identifying and typing RFLPs (including VNTRs) except that the probes are derived from and bind directly to hypervariable repeated sequences of DNA, known as minisatellites, instead of to sequences derived from single, or at most a few, discrete genes (Jeffreys *et al.*, 1985a,b). The minisatellite sequences have no known function and are scattered throughout the genome. Alleles at each locus consist of a variable number of tandem repeats, and the restriction enzymes used to detect alleles cleave the DNA near, but not within, a series of tandem repeats. Therefore, the molecular size differences determined by the variable number of repeats can be detected by agarose gel electrophoresis and Southern blotting with an appropriate probe. In humans, many of these loci have multiple alleles, each at high frequency. Each DNA fingerprint is the sum of the multiple alleles that exist at the many minisatellite loci. Because these repeated sequences are so variable, the probability of any two unrelated individuals having identical DNA fingerprints is almost nil, and even close relatives other than identical twins are not likely to share all detected fragments (Jeffreys *et al.*, 1991). Although the patterns are so complex that it is not possible to trace each fragment as an allele transmitted from one generation to another, the fingerprint patterns of each individual

are so distinct that this technology has supplanted blood group and biochemical genetic markers for many forensic applications. Furthermore, because all bands in an offspring are, in theory, derived from the two parents, DNA fingerprinting is becoming widely used for the purpose of human paternity exclusion.

To date, DNA fingerprinting of nonhuman primates has not been as highly discriminating. Although the nonhuman primate DNA fingerprints are complex, the variability between individuals generally is less than in human subjects. Apparently, the sequences detected by the probes developed for humans are intrinsically less variable in nonhuman primates. Several papers at a recent symposium (Martin *et al.*, 1992) described the development of DNA fingerprinting technologies for a variety of nonhuman primate species. In some instances, the DNA fingerprints were highly informative for the purpose of paternity exclusion, and it appears possible to develop powerful standard protocols for DNA fingerprinting for each commonly used nonhuman primate species. However, it is a consensus that DNA fingerprinting is most appropriately used to complement other types of genetic markers for the purpose of paternity exclusion instead of replacing them.

5. Polymerase Chain Reaction for Indentifying Variation in Single-Copy Sequences

An important advance in molecular technologies is the polymerase chain reaction (PCR) (Saiki *et al.*, 1985; Mullis and Faloona, 1987; also see Oste, 1988; Erlich *et al.*, 1991). This technique enables polymorphic DNA sequences to be typed from small amounts of DNA and circumvents many of the laborious processes involved in RFLP typing. PCR requires knowledge of sequences of nucleotide bases over short segments of DNA flanking the region of interest. Those flanking sequences are used to construct short oligonucleotide primers, which are used to amplify specifically the sequence between them. Thus, the method enables the rapid production of many millions of copies of the sequences of interest. It is then a simple matter to determine by standard procedures (e.g., agarose gel electrophoresis after restriction digestion) which of several variant sequences exist in an individual. Alternatively, the PCR product can be sequenced directly. This approach has largely replaced Southern blotting as a means of typing human DNA sequences that are known to be polymorphic and have known flanking sequences. The use of PCR for research with nonhuman primates is more limited, in part because relatively few genes of any particular species have been sequenced. However, in some cases randomly chosen flanking sequences of human genes can be successfully used to construct primers for PCR amplification of the homologous nonhuman primate DNA (Rogers *et al.*, 1992). The probability of successful amplification can be increased by constructing primers complementary to sequences that are known to be highly conserved among species.

6. PCR for Identifying VNTR and CA Repeat Polymorphisms

VNTR and CA dinucleotide repeat polymorphisms can be detected by PCR using the same techniques as are used for PCR detection of variation in single-copy sequences, provided that flanking sequences needed for primer construction are known. The number of repeats within a $(CA)_n$ block is typically determined by resolving the amplified DNA on polyacrylamide DNA sequencing gels.

The CA dinucleotide repeat polymorphisms are especially powerful as genetic markers because there are a large number of $(CA)_n$ blocks dispersed throughout the genome, and the number of repeats within a given block is highly variable among individuals. In humans, it is estimated that 50,000–100,000 interspersed $(CA)_n$ blocks exist, with the range of n being approximately 15–30 (see Weber and May, 1989; Weber, 1990).

A preliminary investigation of CA repeats in chimpanzees has revealed a high level of polymorphism (Morin and Woodruff, 1992). The number of alleles detected in a survey of 25 *Pan troglodytes troglodytes* was 8 at one locus and 12 at another. Examination of DNA from *P. troglodytes schweinfurthii* revealed 5 alleles in 7 individuals at one locus and 4 alleles in 19 individuals at another locus. It appears that the existence of CA repeat polymorphisms will add a powerful new type of marker for research with nonhuman primates.

7. Mitochondrial DNA Markers

In addition to nuclear DNA markers, variation in mitochondrial DNA (mtDNA) also has contributed significantly to research with nonhuman primates. With few exceptions, mtDNA is inherited in a strictly maternal fashion (i.e., via the mitochondria in the oocyte), so mtDNA markers are not useful for nuclear family studies. However, because mtDNA is more rapidly evolving than nuclear DNA, mtDNA sequence comparisons provide a powerful means of assessing phylogenetic relationships. This topic has been discussed extensively in articles and reviews (Hixson and Brown, 1986; Hayasaka *et al.*, 1988; Harihara *et al.*, 1988; Hasegawa, 1990).

The mitochondrial genes encode some oxidative phosphorylation subunit proteins as well as some tRNAs and rRNAs. A considerable number of disorders of oxidative phosphorylation, by which ATP is produced in mitochondria, are known in humans; some of these are consequences of mutations in mtDNA (Shoffner and Wallace, 1990). Because considerable effort has been expended on the use of mtDNA in phylogenetic research with nonhuman primates, it will be possible to develop these species as models for studying human diseases associated with mtDNA mutations.

E. Technical Considerations

1. Blood Processing and Storage Procedures

Although karyotypic analyses require freshly collected blood from which to initiate short-term lymphocyte cultures, other genetic markers can be typed in appropriately stored blood samples. Even when it is possible to type a marker of interest in freshly collected blood, long-term breeding and research pro-

grams can profit enormously if blood samples are preserved from each individual. This practice enables newly discovered markers to be typed many years into the future, long after the animal itself is dead or absent from the colony.

At those laboratories that use blood group markers, blood is collected in an anticoagulant, and washed red cells are stored in a glycerol solution in liquid nitrogen (Krijnen *et al.*, 1964). Unfortunately, before the stored cells can be used for typing, they must be washed sequentially in a series of solutions with a decreasing ratio of glycerol to saline, until a final wash in saline. Most of the cells are lysed during this process, but enough remain intact to enable the typing procedure to be conducted.

For biochemical genetic marker typing, clots or washed red cells, as well as plasma or serum, are routinely stored. The washed red cells are typically preserved in a glycerol or citrate–ethylene glycol solution and are frozen at −20°C (Cheng and VandeBerg, 1987), but enzymes are more stable and less prone to develop artifactual variations in phenotype when clots are stored at −80°C in the absence of any preservative (unpublished data, J. L. VandeBerg, 1990). Enzymes and other proteins in plasma or serum also are highly stable at −80°C. A novel approach to storage is to seal aliquots of the cells, clots, plasma, or serum in small segments of Tygon tubing (Cheng *et al.*, 1986; Cheng and VandeBerg, 1987). This procedure prevents oxidation from occurring during storage and enables a single tubing segment to be removed without thawing the remaining aliquots whenever a sample is required. Blood stored under these conditions is expected to yield stable marker phenotypes for decades.

White blood cells to be stored for biochemical genetic marker typing can be collected from centrifuged uncoagulated blood as the buffy coat, rinsed with a hypotonic buffer (150 m*M* NH_4Cl, 3m*M* Na_4EDTA, 0.7 m*M* KH_2PO_4, pH 7.0) to lyse erythrocytes, washed twice with phosphate-buffered saline (PBS), suspended in two volumes of PBS, and stored at −80°C in sealed segments of Tygon tubing (unpublished data, J. L. VandeBerg, 1990).

The same procedure can be used for storing white blood cells for DNA marker analysis, although it is not necessary to lyse the contaminating erythrocytes or to seal the cells in Tygon tubing. When it is preferable to isolate the DNA from freshly collected blood, the isolated DNA can be stored indefinitely in buffered aqueous solutions at −20°C (Sambrook *et al.*, 1989).

White blood cells used for MHC typing by serological techniques also can be stored in liquid nitrogen or in a low temperature freezer (−80°C or lower) after isolation by an appropriate technique (Wood *et al.*, 1972; Ruder *et al.*, 1984).

2. Tissue Processing and Storage Procedures

In primate colonies from which some animals are euthanized for research or clinical reasons, it is potentially useful to establish a frozen tissue bank for genetic research purposes. The existence of a tissue bank enables genetic research on many enzymes and other proteins that are not present in blood, and also can provide an almost unlimited source of DNA from each animal for molecular genetic studies. Because of the minimal expense and effort required to preserve tissues indefinitely and their utility for biochemical and molecular genetic research, establishment of a tissue bank should be considered even for colonies at which an immediate need for such research is not anticipated.

Tissues should be collected at the time of necropsy as soon as possible after the death of the animal. Small samples (typically 1, 5, or 10 g) can be wrapped in aluminum foil or placed in a small vial, quick frozen with dry ice or liquid nitrogen, and stored permanently at −80°C. For protein electrophoresis, a small chip (typically 50–100 mg) can be removed from a frozen sample for homogenization, whereas for DNA extraction about 1 g of tissue is typically used. However, it is possible to work with smaller amounts in instances where the sample size is limited.

3. Validation of Genetic Markers

Phenotypic differences are sometimes assumed to reflect genetic differences even in the absence of family data. Such assumptions usually are based on analogy with species in which the genetic nature of similar phenotypes was confirmed by analyses of pedigreed family members. If the data are derived from a population believed to be randomly mating and if the observed phenotypic frequencies fit those that are expected under Hardy–Weinberg equilibrium, the assumption of Mendelian inheritance is strengthened. However, even under these circumstances, many such phenotypic differences have turned out to be epigenetic and thus are not inherited as Mendelian traits. Partial degradation of the protein under investigation in some, but not all, samples can easily lead to this erroneous conclusion; the literature contains examples of such data. In some cases, extensive analyses were conducted using "markers" that reflected artifactual differences rather than true genetic differences between individuals.

Because of this potential for error, it is important that newly discovered genetic markers be verified through analyses of families for which accurate pedigrees exist. A demonstration of how this procedure was used in defining and verifying the first biochemical genetic markers in squirrel monkeys was provided by VandeBerg *et al.* (1990a).

IV. GENETIC MANAGEMENT APPLICATIONS

As nonhuman primates have become more important for biomedical research purposes, the supply of wild-caught individuals of many species has been severely curtailed or terminated altogether. This trend unquestionably will continue, possibly at an increasing rate. Consequently, to ensure the long-term availability of nonhuman primates, geneticists have begun to develop strategies for genetically managing captive breeding colonies.

A. Species and Subspecies Identification

Some species and subspecies of nonhuman primates are poorly defined and poorly characterized. In these instances, the risk of inadvertently establishing mixed breeding groups or assigning mixed groups to experimental protocols is high. The consequences can be disastrous for breeding programs and for valid interpretation of experimental results.

A good example of the potential problem is illustrated by squirrel monkeys, which comprise the genus *Saimiri*. Based on morphological characteristics, squirrel monkeys have been classified as four species and five subspecies (Hershkovitz, 1984). Some of these types are morphologically similar and hybrids can be produced so classification by inspection alone can be unreliable. All squirrel monkeys have a chromosome number of 2N = 44, but pericentric inversions have occurred in some chromosomes during evolution, resulting in karyotypic variation among some species and subspecies. Therefore, in instances where two types of squirrel monkeys have distinguishable karyotypes, cytogenetic analysis is believed to be capable of distinguishing between them and discriminating them from first generation hybrids. The power of cytogenetic analysis to help in verifying species and subspecies identity was illustrated by a karyotypic analysis that detected misclassification of 34.5% of imported squirrel monkeys classified, on the basis of morphological criteria, as *S. boliviensis peruviensis* (Ariga *et al.*, 1978).

If different chromosomal types of squirrel monkeys (or any other species) are inadvertently combined into a breeding group, a decline in reproductive success is likely to occur due to mismatched chromosomes (e.g., pairs heterozygous for inversions or translocations) in the hybrids. Recombination between mismatched chromosomes during meiosis in hybrid progeny leads to a proportion of gametes (up to 50% for a single chromosomal mismatch) that have chromosomal duplications or deficiencies. Such gametes are likely to cause embryonic death.

An even more serious consequence of the inadvertent mixing of squirrel monkey types occurs when experimental groups are formed, because the different types of squirrel monkeys exhibit major differences in their genetically controlled physiological characteristics (reviewed by Abee, 1989). Thus, experimental results obtained from groups that contain more than one subspecies or that contain hybrid individuals are likely to lead to erroneous interpretations.

Unfortunately, not all squirrel monkey types can be distinguished karyotypically. Furthermore, in instances where two taxa differ by a single pericentric inversion, half of the second generation hybrids and backcross hybrids will have karyotypes indentical to one of the parental types. Biochemical genetic markers, used in conjunction with the cytogenetic markers, can provide higher discriminating power in these instances (VandeBerg *et al.*, 1990b).

These concerns about accurate species and subspecies identification of nonhuman primates used in breeding and research programs apply to many genera of New World primates and even to some genera of Old World primates. For example, difficulty in ascertaining the species and subspecies of macaques originating from the Sulawesi (Celebes) islands was described by Lacy *et al.* (1988), who also used biochemical markers to help in discriminating among different taxa.

B. Parentage Identification

Animals with known pedigrees are essential for genetic research. Pedigree records also are required to enable minimization of inbreeding in long-term breeding programs. The gradual recognition of the potential value of pedigreed animals for research and genetic management purposes has led to the maintenance of pedigree records at increasing numbers of primate breeding facilities.

Some initial attempts to establish paternity records relied on the assumption that the dominant male in a breeding group was responsible for siring all of the progeny. However, several investigations with genetic markers revealed that numerous offspring were sired by subordinate males (Duvall *et al.*, 1976; Curie-Cohen *et al.*, 1983b; Stern and Smith, 1984; Inoue *et al.*, 1992). In some instances, the dominant male sired fewer than half of the offspring born.

Whether these results are representative of the relationship between rank and reproductive success in natural populations or are because of unnatural conditions of captivity is not known. However, support for the latter hypothesis was obtained from an investigation of wild *Macaca fascicularis*, which exhibited a high correlation between the rank of males and their reproductive success (de Ruiter *et al.*, 1992)

Analysis of genetic markers can determine the sires of some progeny in multi-male breeding groups by excluding all but one of the adult males (e.g., see Smith, 1980; Stone *et al.*, 1981). The power of this approach diminishes rapidly as the number of potential sires increases (Dyke *et al.*, 1990), and of course it depends on the extent of heterogeneity in marker phenotypes of both the dams and the potential sires. When it is possible to select the adult members for a breeding group on the basis of their marker phenotypes, the power of paternity exclusion can be increased substantially. As VNTR and DNA fingerprinting techniques become established for each species of nonhuman primates, the construction of accurate pedigrees for multi-male breeding groups will be facilitated. However, it is the consensus, based on scientific and economic considerations, that DNA markers are most appropriately used in addition to rather than in place of protein markers (Martin *et al.*, 1992).

Under the current limitations, many colony managers have established single-male breeding groups. However, even in these cases it is essential to conduct genetic marker analyses to verify pedigrees because (1) sometimes females are impregnated by juvenile males which were thought to be too young to reproduce, (2) pregnancy may result from mating with males in

adjacent cages separated by a mesh fence, and (3) sometimes errors are made in recording family data or caging histories. Genetic monitoring practices will detect many of the pedigree errors that arise for these or other reasons. Further discussion of this topic and calculations of the power of specific markers to detect pedigree errors are presented in VandeBerg (1992).

Maternity can usually be determined accurately in nonhuman primate breeding groups because of the close bond between mother and infant. However, recording errors occur in maternity records as frequently as in paternity records; in rare instances, mothers who delivered babies at about the same time have been known to swap them (e.g., see Curie-Cohen *et al.*, 1983a; VandeBerg *et al.*, 1990a).

Even the best genetic monitoring system will not detect all pedigree errors because the number of markers for any one species of nonhuman primate is limited. In practice, at least until such time as an excess of markers is available, the best approach is simply to type all progeny and their parents for whatever polymorphic markers are practical to type for that species.

Highly accurate pedigree records derived by genetic monitoring are extremely valuable for genetic research and are very useful for other areas of research as well. For example, it would not have been possible without genetic markers to discover that the dominant male does not necessarily sire most of the offspring in a troop. Furthermore, certain types of research projects require animals that have been genetically characterized for one marker or for an entire constellation of markers, so genetic monitoring of a colony provides a "library" of genotypes from which to draw those required for specific research projects.

C. Minimizing Inbreeding

Data derived from a large number of mammalian species indicate that inbreeding is associated with reduced reproductive success and infant survival (Wright, 1977; Ralls *et al.*, 1979; Ralls and Ballou, 1983). The collective detrimental effects of inbreeding are called "inbreeding depression." Inbreeding can be avoided if accurate pedigree records are maintained and if sufficient numbers of animals are available to enable breeding programs that preclude matings between relatives.

However, economic considerations dictate that some populations consist of a large number of breeding females and a few males. The risk of inbreeding in these instances is high because the population eventually will contain many half-sibs and some full sibs, as well as many parent–offspring combinations. Indeed, since the reproductive span of a male is typically several times longer than the age to sexual maturity, a sire could conceivably mate with his own daughters, with granddaughters produced by that mating, and even with great-granddaughters. It has been argued that inbreeding occurs in some primate species under natural conditions without causing reproductive decline. However, under natural conditions there is considerable gene flow into and out of breeding groups as a result of migration (discussed by Melnick, 1988), and the duration of time during which an animal is highly reproductive is likely to be much shorter than that which is possible in many captive situations (discussed by Altmann and Altmann, 1979). In addition, it is apparent that incest avoidance occurs in at least some species of nonhuman primates when alternative mates are available (for example, see Wickings and Dixson, 1992). Furthermore, the colony manager's goal of maximal long-term reproductive success of a population is not necessarily achieved under natural conditions.

A variety of strategies can be employed to reduce the rate of inbreeding in instances of multi-male breeding groups: (1) Increasing the generation time by maintaining breeders throughout their useful reproductive lives will reduce the rate of inbreeding on a per year basis. Under this strategy, and in a population of fixed size, all juveniles are harvested for research purposes except when a breeder must be replaced. This strategy does not reduce the rate of inbreeding on a per generation basis. (2) In some instances, it may be possible to replace the breeding age males periodically by introducing unrelated animals without undue risk of social disruption. This strategy mimics the natural condition for many species, in which males leave their natal troops and seek entry into other troops. In situations where this strategy is not practical because of social structure, it may be possible to introduce males into breeding groups as infants by cross-fostering (Smith, 1986). (3) Equalizing as nearly as possible the genetic contribution of all breeders to the next generation will reduce the level of inbreeding in a population. This strategy is practical when applied to dams, but not to sires in multi-male breeding groups unless genetic markers are used to determine paternity. In instances where paternity assignment is possible, sires can be removed after contributing a specified number of progeny to the next generation of breeders, or alternatively their progeny can be selectively removed to equalize the genetic contributions of the sires. (4) More nearly equalizing the sex ratio will reduce inbreeding, provided that measures are implemented to ensure that additional males do in fact sire offspring. A more detailed consideration of inbreeding in nonhuman primates and strategies for its reduction have been provided by Smith (1980).

Unfortunately, each of the strategies that is potentially effective for reducing the level of inbreeding has both research and economic consequences, which must be carefully considered in making management decisions. Each breeding situation is unique, and judgment regarding cost in relation to likely benefit is critically important.

D. Maintaining Genetic Variability

The extent of genetic variation in any population is associated with its long-term survival. In cheetahs (O'Brien *et al.*, 1983, 1985) and in inbred strains of animals, variability is

reduced or absent, and infant mortality and reproductive failure are high. In most instances where genetic variability is severely reduced, such as during the development of inbred strains, the population becomes extinct.

Most natural populations of nonhuman primates exhibit genetic variability at approximately the same levels as exist in humans and most other mammalian species in nature. However, in comparison with natural populations, captive breeding colonies of nonhuman primates are generally quite small, numbering in the dozens, scores, or hundreds. As genes are transmitted from one generation to another, chance alone causes the loss of rare alleles from small populations. As approach to reducing the loss of rare alleles has been implemented by Dyke *et al.* (1987). They developed a computer program as an aid in selecting juvenile baboons for harvest, and those for future breeding, on the basis of their biochemical marker alleles in addition to other pertinent characteristics. Those animals with the rarest constellation of alleles at the various loci typed are preferentially saved for breeding. This simple procedure not only preserves variability for general management purposes, but also can facilitate genetic research as well as paternity determination.

E. Management of Blood Group Incompatibilities

Maternal–fetal incompatibility for the Rh blood group system (i.e., erythroblastosis fetalis) was a serious problem in human medicine prior to the development of the passive immunization procedures routinely used to prevent Rh^- mothers carrying Rh^+ fetuses from developing an anamnestic immune response of potential detriment to subsequent fetuses. Although the human Rh blood group was discovered with antibodies developed by injecting rhesus red blood cells into rabbits, rhesus and most other monkeys do not spontaneously develop clinical disease as a consequence of maternal–fetal blood group incompatibilities (Socha and Moor-Jankowski, 1986; Treichel, 1987). Maternal alloantibodies engendered by transplacental immunization do coat the newborn's erythrocytes, but do not mediate their elimination as occurs in humans. Even the great apes do not appear to be susceptible to spontaneous erythroblastosis fetalis, although the intentional immunization of mothers against blood group antigens that are present in subsequent fetuses can lead to the disease (Wiener *et al.*, 1977). Therefore, if female apes are immunized against blood from other individuals, their subsequent mates should be selected on the basis of blood types, i.e., mates should not possess highly immunogenic blood group antigens against which the mother has been immunized (Socha and Moor-Jankowski, 1986). In instances where matings that might lead to maternal–fetal incompatibility occur, pregnancy can be monitored and erythroblastotic babies can be saved by exchange transfusion (Socha and van Foreest, 1981).

Only one species of nonhuman primate is well documented to exhibit spontaneous erythroblastosis fetalis on a frequent basis: the marmoset *Saguinus nigricollis* (Gengozian *et al.*, 1966). The investigators who made this observation speculated that the disease could be a consequence of a trabecular rather than a villous placenta in this species, enabling more intimate contact of maternal and fetal tissues. They also suggested that consistent fraternal twinning and blood chimerism in this species could predispose to this disease. Whatever the underlying cause, the documentation of the problem in marmosets suggests the value of testing the serum of multiparous females against the erythrocytes of their potential mates for evidence of prior immunization against highly immunogenic blood group antigens.

Blood group incompatibilities also are a concern with regard to the potential for blood transfusion reactions. Since the apes exhibit A–B–O antigens on their erythrocytes and contain anti-A and anti-B isoagglutinins in their plasma, even the first transfusion of A–B–O incompatible blood will cause severe and dramatic reactions (Socha, 1986). Even in primate species that exhibit only simian-type antigens on their erythrocytes, multiple transfusions can, on rare occasions, cause reactions due to antibodies engendered by earlier transfusions. Even though such reactions occur infrequently, matching of donors and recipients for therapeutic transfusions is clinically important. In experiments involving a series of transfusions between intentionally mismatched baboons and rhesus monkeys, the half-life of the transfused cells was significantly reduced when corresponding antibodies were present in the serum of the recipient; in some cases, the half-life was so short that the transfusion was therapeutically worthless (Socha *et al.*, 1982).

V. DEVELOPMENT OF ANIMAL MODELS

The high degree of similarity between humans and nonhuman primates, due to their close phylogenetic relatedness, makes nonhuman primates ideally suited to serve as models for many normal and pathological human conditions. The development of the various types of genetic markers and the construction of accurate pedigrees make it possible now to exploit nonhuman primates effectively as genetic models for basic and applied research. This section provides some examples of nonhuman primate models for genetic conditions and summarizes some basic concepts and principles of genetic applications in the development of animal models.

A. Single Gene Diseases

Despite the enormous number of human diseases caused by single gene defects, a 1986 review of the literature revealed no instances in which research with nonhuman primates had revealed a well-defined hereditary disease controlled by a single gene (Benirschke, 1986). It is possible that nonhuman primates have an extremely low incidence of deleterious mutations, by comparison with humans; but it seems more likely that the ab-

sence of known hereditary diseases is, at least, in part, a result of limited study. Hereditary diseases with an incidence as low as one in a million are readily detected in human subjects because of the number of people and the modern health care systems throughout the world. The number of nonhuman primates in breeding colonies is minuscule by comparison, and rare hereditary diseases that do exist and cause infant mortality are unlikely to be diagnosed.

Most inherited single gene diseases are a consequence of homozygosity for a recessive gene. The frequency of a rare recessive gene in any population that is randomly mating with respect to that gene is estimated as the square root of the frequency of affected individuals. Therefore, if the frequency of affected individuals was 1/40,000 (0.000025) in a breeding population, the frequency of the recessive gene in that population would be 1/200 (0.005). For rare genes, the frequency of carriers is approximately twice the gene frequency; for this example $2 \times 0.005 = 0.01$. Many genes for inborn errors of metabolism have frequencies of 0.005 or higher in some human populations. Thus, an effective approach to identifying single gene defects in nonhuman primate populations would be to subject the individuals to screening tests designed to detect human carriers. If a single carrier is found in a nonhuman primate species, selective breeding would enable the gene to be established in a group of animals dedicated to research on that genetic defect.

Precisely this approach was used in the last few years to develop the first single gene disease model in a nonhuman primate species. The disease is familial hypercholesterolemia (FH), which is caused by a deficiency in the low density lipoprotein (LDL) receptor. About 1/500 humans are heterozygotes for a gene that causes LDL receptor deficiency and these heterozygotes have elevated LDL cholesterol levels and early onset atherosclerosis. Homozygotes for this gene have heart attacks as early as age 2, and almost inevitably by age 20. A survey of about 100 rhesus macaques revealed a mother–son combination with elevated LDL cholesterol and other lipoprotein features characteristic of heterozygosity for LDL receptor deficiency in humans (Scanu *et al.*, 1988; Neven *et al.*, 1990; Hummel *et al.*, 1990). Further analysis at the DNA level revealed that these two individuals and another progeny born later to the same dam were indeed heterozygous for LDL receptor deficiency (Hummel *et al.*, 1990). The rhesus family is currently being expanded to provide larger numbers of individuals, including homozygotes, for experimentation. Accuracy of the pedigree records for this colony is being ensured by the use of genetic markers (W.H. Stone, personal communication, 1990).

This approach for developing nonhuman primate models of single gene diseases is practical for any disease in which screening for heterozygosity can be conducted economically on a large scale.

Another approach to detecting carriers would be to target the parents of any infant suspected of having a metabolic defect, under the assumption that both parents were heterozygous for a recessive gene that caused the defect in their presumed homozygous progeny. The strategy would be to acquire as much information as possible about the pathology of the disorder, to relate that information to human single gene diseases, and then to screen the two parents for heterozygosity for all genes that were considered candidates on the basis of the pathological results.

Another approach for developing nonhuman primate models for research on human single gene diseases is to perturb the normal physiological processes of the animal in a way that affects gene expression. For example, β-thalessemia is caused by one or another genetic defect in the β-globin gene, causing β-globin deficiency and resultant hemoglobin anomalies. If the fetal hemoglobin gene (γ-globin) could be turned on in individuals suffering from β-thalessemia, it is expected that the clinical symptoms could be relieved. It has been discovered that hematopoietic stress of baboons causes activation of the γ-globin gene and production of fetal hemoglobin ($\alpha_2\gamma_2$) (DeSimone *et al.*, 1978). The magnitude of the fetal hemoglobin response is apparently controlled by genetic factors (DeSimone *et al.*, 1980). Thus, the baboon serves as a model to investigate the molecular mechanisms that control the activation and repression of fetal hemoglobin genes (Lavelle and DeSimone, 1989) and to explore the feasibility of treating β-thalessemia by activation of the γ-globin gene.

B. Common Multifactorial Diseases

In contrast to the lack of nonhuman primate models for diseases caused by single gene defects, nonhuman primates are a rich source of models for common multifactorial diseases, including atherosclerosis, dyslipoproteinemias, hypertension, diabetes, obesity, alcoholic cirrhosis, and anxiety disorders (Stone *et al.*, 1987b). These chronic diseases are mediated by interactions among genetic factors (i.e., multiple genes) and environmental factors. Only in the last two decades has it become generally recognized that genetic variation has a major role in determining which individuals will develop one or another of these diseases and what the severity and pattern of progression of the disease will be. Nonhuman primates are ideally suited as models for these common diseases because the pathophysiologic processes involved are complex and exhibit a high degree of variation among mammalian orders. Because of the close phylogenetic relatedness of humans and nonhuman primates, many of these processes are sufficiently similar in both groups to enable one or another nonhuman primate species to serve as a good model at a level of detail not possible with nonprimate models. Because these chronic diseases are responsible for most of the disabilities, deaths, and health care expenses in industrialized societies, the importance of nonhuman primates in biomedical research has escalated as their role as potential models has become better appreciated.

The immediate goals in research on multifactorial diseases are (1) to identify and characterize the genes responsible for

differential susceptibility, (2) to determine the mechanisms by which the genes control susceptibility, and (3) to determine how the genes interact with one another and with diet and other environmental factors. Accomplishment of these goals and their application to human subjects will enable the identification of genetically susceptible individuals early in life by analysis of genetic markers in blood samples, and the provision of individually tailored dietary and other life-style recommendations. Eventually, preventative therapies can be developed to confer resistance to individuals who are naturally susceptible.

A general strategy for initiating genetic research on a common disease is to determine the genetic contribution to quantitative variation in characters believed to have a role in the disease process and to identify inherited discrete genetic variants that may be responsible for a portion of that quantitative variation. These genetic markers can be polymorphisms at the level of the enzymes and other proteins believed to be pertinent to the quantitative character or they can be individual DNA sequence differences in "candidate genes" that encode those proteins or regulate their production or biological function. Atherosclerosis is an example of a common disease for which nonhuman primate models have been used extensively and are considered for many purposes to be superior to other animal models (reviewed by Vesselinovitch, 1988). The quantitative characters of greatest interest in regard to atherosclerosis are concentrations of plasma lipid components and proteins that affect plasma lipid components. These include total cholesterol, low density lipoprotein cholesterol, high density lipoprotein cholesterol, specific apolipoproteins, specific cellular receptors of lipoproteins, and enzymes involved in lipoprotein metabolism. The candidate proteins in which discrete variation is sought are the specific apolipoproteins and enzymes involved in cholesterol metabolism; variation at the level of the proteins is detected by electrophoretic and isoelectric focusing techniques. Genetic differences in the candidate genes at the DNA level are detected as RFLPs.

Large pedigreed families enable the inheritance patterns of the quantitative characters to be assessed by segregation analysis (MacCluer, 1989). The primary goal is to identify characters whose concentrations are determined to a large extent by one or two genes. Such genes are called "major genes," where "minor genes" exert a small influence on a quantitative character.

When segregation analysis indicates that a major gene is present, the next step is to conduct quantitative trait linkage analysis (MacCluer, 1989). This procedure involves searching for a relationship in transmission from one generation to the next between the level of the quantitative character and a discrete genetic marker. The markers most likely to yield positive results are the variants in candidate genes or proteins, but all genetic markers of any type are potentially useful. When a quantitative character and a discrete genetic marker tend to be inherited together in families, then it is inferred that a major gene influencing the quantitative character is closely linked on the same chromosome to the gene for the marker.

The strategy for identifying the precise gene of interest (so its mechanisms of action can be investigated) depends, at least in part, on the results of the linkage analysis. If a candidate gene is implicated as the major gene, then detailed investigations of the structural and functional characteristics of its allelic forms can proceed using the full repertoire of cloning, sequencing, and other molecular biological techniques; *in vivo* metabolic studies of animals with the various genotypes under carefully controlled dietary and other environmental conditions; and in some cases *in vitro* studies with cultured cells.

If none of the candidate genes used in the linkage analysis is implicated as the major gene or if the approaches described in the foregoing paragraph fail to confirm a candidate gene as the major gene, other approaches must be used to identify the major gene. An attractive and powerful approach involves exploiting the high resolution human gene map which will be available soon in concert with lower resolution gene maps that are being developed for some nonhuman primate species (Lalley *et al.*, 1989). Because gene linkages are highly conserved between humans and nonhuman primates, it will be possible to examine the high resolution human map in identify candidate genes that are close to the marker gene, which in the nonhuman primate species exhibited linkage with the major gene of interest. The candidate genes identified in this manner can then be examined in the nonhuman primate families for evidence of genetic variants that are responsible for the major gene effect.

C. Gene Therapy

One of the most exciting frontiers of genetic research is the development of methods for gene replacement therapy. Initial efforts in gene therapy will be focused on treating life-threatening diseases caused by inborn errors of metabolism (genetic deficiencies in enzymes or other proteins). The approach will be to insert the normal, functional gene *in vitro* into cells derived from the patient, and then to replace the cells back into the individual from which they were derived. Major problems that need to be overcome are getting enough cells with the corrective gene into the patient to enable a sufficient amount of the missing gene product to be produced, getting those cells to proliferate in the patient on a permanent basis, getting the missing gene product transported to the sites where it is required in the body, and accomplishing all of the just-mentioned problems before permanent damage occurs. Results obtained to date are encouraging that these problems can be overcome, at least for some inherited protein deficiency diseases, but much research remains to be done.

Nonhuman primates have a critical role in the development of such methods and in testing them for effectiveness and safety. The efficacy and safety of gene therapy will depend on the interactions of many complex biological systems, and those procedures that are developed in nonprimate models are unlikely to yield precisely the same results in primates. For ex-

ample, an efficient retroviral-mediated gene transfer protocol established in a mouse model (Eglitis *et al.*, 1985), when applied to a nonhuman primate species, resulted in sepsis or persistent bleeding from thrombocytopenia (Eglitis *et al.*, 1987). Only when the procedures for transferring the gene into bone marrow cells were changed substantially was it possible to succeed in a gene therapy experiment with nonhuman primates (Eglitis *et al.*, 1987). Nonhuman primates also have been used to develop procedures that increase the efficiency of retroviral-mediated gene transfer and expression in bone marrow progenitors (Wieder *et al.*, 1991).

Another safety concern is the retroviral vectors, which are currently the most widely used vehicles for delivering corrective genes into cells. The retrovirus DNA might interact with endogenous DNA in the treated cells in ways that alter cell growth characteristics, leading to cancer. Because different retroviruses might be used for gene transfer in primates than in other animals and because primate DNA sequences are quite different from those of other mammals, the potential adverse interaction between the retroviral and host DNA should be examined in nonhuman primate models.

It is not necessary that nonhuman primates actually have a protein deficiency disease to serve as a model for this research. Rather, genes that encode biochemical genetic markers can be inserted and their expression monitored electrophoretically. A marker gene could be derived from the same species used in the gene therapy research or it could be a human gene whose protein product has an electrophoretic mobility that differs from that of some protein in the nonhuman primate species.

Most attention has focused on using the patient's own bone marrow cells as the vehicle for transporting corrective genes into an individual because they are histocompatible and relatively easy to collect, manipulate, and reintroduce into that individual. In addition, some deficiency diseases exert their primary clinical effects via the hematopoietic system. One of these conditions is deficiency of adenosine deaminase (ADA), which causes one form of severe combined immunodeficiency disease. Most individuals with ADA deficiency die by 2 years of age. The apparent metabolic basis of the disease is the accumulation of toxic levels of 2'-deoxyadenosine and 2'-deoxyadenosine triphosphate in lymphocytes, leading to nonfunctional T cells and B cells. Eglitis *et al.* (1987) used retroviral vectors to introduce human ADA genes into bone marrow cells from rhesus and cynomolgus macaques and returned the cells to their donors. The cells did produce human ADA in six of the eight monkeys analyzed, establishing the feasibility of this approach to gene transfer in primate species.

Baboons and rhesus monkeys have been used in preliminary experiments to assess the feasibility of transferring hematopoietic stem cells into fetuses. These experiments involved transferring stem cells from a donor to a recipient rather than replacing genetically modified cells back into the individual from which they were derived. In the baboon model, the donors were adults, and the donors and recipients were selected to be homozygous for alternative electrophoretic variants of glucose-phosphate isomerase (GPI) (Roodman *et al.*, 1988). The effectiveness of the engraftment could be assessed in all blood cell types as a ratio of donor to recipient GPI. Engraftment did occur in some fetuses, but the grafts did not persist, possibly because of the immune reaction mounted by the fetus against the donor cells and possibly because the fetal stem cells proliferated faster than the engrafted adult stem cells during late fetal development. In the rhesus monkey model, the donors were fetuses, and engraftment was assessed by karyotypic analysis of peripheral blood leukocytes and bone marrow of recipients for which the donor had been of the opposite sex. In these monkeys, the grafts have persisted on a permanent basis (Harrison *et al.*, 1989). The results of these experiments suggest that it may be possible to develop gene therapy methods for treating fetuses diagnosed with deficiency diseases by amniocentesis. The implications are significant because many single gene diseases already have had irreversible effects by the time of birth.

Future research on gene therapy with nonhuman primate models will play an important role in these rapidly developing technologies, not only for establishing the technical approaches for successful gene therapy, but also for evaluating the potential risks and benefits under genetic and physiologic conditions most nearly like those of humans and over a lifespan of several decades.

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CHAPTER 9

Reproductive Biology

Andrew G. Hendrickx and W. Richard Dukelow

I. INTRODUCTION

Although the overall number of nonhuman primate species used in reproductive research has declined since the early 1970s, there are still large numbers utilized for this purpose. Among them are several prosimians (lemurs and bushbabies), New World species (squirrel monkeys, marmosets, tamarins), Old World species (rhesus, cynomolgus, pig-tailed macaques, baboons, African green monkey), and apes (chimpanzees). The rhesus monkey has been, by far, the most commonly used species for research in reproductive biology until recently, as they have been replaced to a large extent by the cynomolgus or long-tailed macaque. This shift in choice of species was brought about largely by the exportation ban of the rhesus by the Indian government and the ready availability of the long-tailed macaque from Indonesia and the Philippine Islands. In considering the contents of this chapter, we primarily utilized the data available for the more commonly used species and present informa-

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tion from the lesser used species only in order to emphasize a point. Furthermore, the species that are considered in some depth are those that, to some extent, are being bred under laboratory or captive conditions and are obtained from self-sustaining populations; therefore the use of threatened or endangered species is minimized or eliminated. No attempt was made to present a comprehensive bibliography; the references presented are often only several of a larger number of pertinent publications. A small number of comprehensive reviews are given under most subtopics: puberty, the ovarian (estrous and menstrual) cycles, pregnancy, parturition, lactation, reproductive senescence, spermatogenesis, and male reproductive tract fluids. It is essential to consider reproduction as an integrated process; each event should not be viewed as an isolated occurrence. This chapter also emphasizes the current knowledge of the major events in the reproductive life cycle of both sexes.

II. PUBERTY

Puberty encompasses the period of development during which the first changes in reproductive hormones are evident and the capacity of the individual to reproduce successfully is achieved. The events surrounding this period of development appear to be similar in most primate species, although critical information for a number of the lower forms is not readily available. Because of the complexity of these events, several hypotheses have evolved which relate to the factors which control puberty onset, most of which are beyond the scope of this chapter. The reader is referred to reviews for further information (Plant, 1988; Plant *et al.*, 1989; Steiner *et al.*, 1983; Terasawa *et al.*, 1983).

Few endocrinological studies have been carried out in the prosimians and New World species relating to this event. In Old World primates and anthropoid apes, puberty in the female is marked by the onset of menstruation and first ovulation, which will usually not occur simultaneously, and by the initiation of increased testicular size and spermatogenesis in the male. Major changes in body weight and size (the "adolescent growth spurt") can occur before, during, or after the initiation of cyclicity in females and is unique to the species (Hobson *et al.*, 1980). In many cases, body weight changes are the only method for detecting puberty onset.

A. Female

1. Prosimians and New World Species

First dates of breeding and conception are known for a number of these species (see Table I). Puberty occurs in the greater bushbaby between 10 and 18 months of age with the first conception occurring from 15 to 18 months (Hendrickx and Newman, 1978). The slender loris reaches sexual maturity at approximately 10 months of age and at least one conception occurs at 1 year of age (Izard and Rasmussen, 1985). In the slow loris, females copulate between 18 and 24 months (Izard *et al.*, 1988). Among the lemurs, the ruffed lemur conceives at 20–21 months with cyclicity reported as early as 9 months in captivity (Brockman *et al.*, 1987). The ruffed lemur is the only lemur that exhibits cyclical vaginal dilation and constriction in a fashion similar to the galagos and other prosimians (Boskoff, 1977). The mongoose lemur conceives at approximately 26 months of age (Schaaf and Stuart, 1983).

In the squirrel monkey, marked changes in hormonal levels exist between 1 and 3 years of age with puberty occurring in the females at approximately 2.5 years (Coe *et al.*, 1981). If the females are small for their age, puberty can be delayed until 3.5 years of age but normally will be associated with the environmental cues that initiate the breeding season. There is a strong effect of body weight on hormone secretion and the onset of puberty in this species (Coe *et al.*, 1985). Among marmosets, regular ovulatory cycles do not occur until 14–18 months of age and sexual maturity is not attained until 20–24 months, with considerable variation between individuals. Tamarin females that mature within their family groups do not show cyclical patterns or reproduce; therefore, the age of sexual maturity is difficult to determine. Histological data suggest that tamarins reach sexual maturity at approximately the same time as marmosets (Epple and Katz, 1983). Howler monkeys have been reported to show first estrus at 34–37 months and at least one birth was recorded in a 43-month-old female. Owl monkeys are reproductively mature by 18–24 months of age (Dixson, 1983).

TABLE I

PUBERTY IN FEMALE NONHUMAN PRIMATES: PROSIMIANS AND NEW WORLD SPECIES

Species	Age	
	Puberty (months)	Sexual maturity (years)
Prosimians		
G. crassicaudatus	10–18	15–18 months
L. catta	—	1.5
L. mongoz	—	26 months
L. tardigradus	10	1.0
M. murinus	—	0.8
N. coucang	—	17 months
P. potto	—	1.0
Propithecus	—	2.5
V. variegata	9	1.5–2.0
New World species		
A. trivirgatus	—	18–24 months
Alouatta	34–37	3–4
Callithrix	14–18	1.5–2
Cebus	—	3–4
S. sciureus	2.0–3.0	2.5–3.5
Saquinus	14–18	1.5–2

TABLE II

PUBERTY IN FEMALE NONHUMAN PRIMATES: OLD WORLD SPECIES AND THE GREAT APES[a]

Species	Age (years)		
	Menarche	Sexual maturity	First birth
Old World species			
C. aethiops	2.5	—	—
C. talapoin	3–4	4	—
E. patas	—	2	3–3.5
M. mulatta	2–3	2.6–3.5	3–5
M. fascicularis	2.0–3.0	3.0–3.4	3.7–5.0
M. arctoides	2.5–3.5	3.0	3.4–4.9
M. cyclopis	2.5–3.5	3.0	—
M. nemestrina	2–4	~3.4	3.9
M. radiata	—	3.5	4.1
M. sylvanus	—	~4.0	4.9
P. entellus	2–3	—	3–4
Papio	3–4	3–4	3.5–5
Great apes			
P. troglodytes	7–11	8	10–13
P. pygmaeus	7	6–8	~12
G. gorilla	6–8	6–7	10

[a]Adapted from Butler (1974); Nieuwenhuijsen *et al.* (1987); and Spies and Chappell (1984).

2. Old World Species and the Great Apes

a. MENARCHE. Menarche, the onset of menstrual cyclicity, is regarded as an overt sign of the initiation of puberty, although endocrine profiles indicate significant hormonal changes 6 to 8 months prior to detection of this event (Terasawa *et al.*, 1983). In most species studied, the initial length of menstruation is sporadic, frequently for several months to a year. Menarche has been reported to occur in the rhesus (*Macaca mulatta*) as early as 2 years of age (van Wagenen and Catchpole, 1956; Foster, 1977), although the mean range reported overall was 2 to 3 years. Other species studied include *M. cyclopis* (3 years ± 6 months) (Peng *et al.*, 1973), *M. arctoides* (2.5–3.5 years) (Nieuwenhuijsen *et al.*, 1987), *M. fascicularis* (2–3 years) (Honjo *et al.*, 1984), *C. aethiops* (~2.5 years) (Bramblett *et al.*, 1975), *P. cynocephalus* (3–4 years) (Gilman and Gilbert, 1946; Glassman *et al.*, 1984), *P. entellus* (2–3 years) (Winkler *et al.*, 1984), chimpanzee (7–11 years) (Young and Yerkes, 1943; Tutin, 1980), and gorilla (6–8 years) (Harcourt *et al.*, 1980) (see Table II).

In the rhesus, several reports have indicated large variations in the intermenstrual intervals at puberty onset. Of the 48 pubescent females studied by Resko *et al.* (1982) (24 at the Oregon Regional Primate Center and 14 at Wisconsin), an average duration of the first intermenstrual interval was greater than 90 days (Table III). Menarche was observed in females from the Oregon group at 123.3 ± 6.6 weeks and at 133.0 ± 3.1 weeks for Wisconsin-housed animals. Ovulatory capability was monitored in 14 animals via hormonal analyses during the 12 intermenstrual intervals following menarche. It was noted that none of the females ovulated during the first interval, 50% ovulated by the 8th interval whereas 90% ovulated by the 12th interval. The average age at initiation of the 12th interval was 207 ± 3.7 weeks. These data suggest that follicular growth occurred without a pituitary-derived ovulatory stimulus. In addition, the results supported the "adolescent sterility" hypothesis (the interval between menarche and first ovulation) initially proposed by Young and Yerkes (1943). These authors reported a mean age for menarche onset in the chimpanzee as 8 years 11 months, with mean cycle lengths of 50 ± 4.4 days (first month). This was compared to a cycle length of 35 ± 0.8 days which was noted during the second half of the 7th year of age. Other reports in great apes have confirmed observations regarding the initiation of puberty (7–11 years) in these species (Coe *et al.*, 1979; Winter *et al.*, 1980).

Studies in the wild have shown that *P. troglodytes* females show subtle changes in sex skin in the 1 to 3 years prior to the development of full-sized swellings and menarche (Harcourt *et al.*, 1980; Tutin, 1980). Changes in the appearance of sex skin prior to menarche have also been reported for *M. mulatta* (Wilen and Naftolin, 1976), *M. nemestrina* (Erwin and Erwin, 1976), *E. patas* (Rowell, 1977b), *P. cynocephalus* (Castracane *et al.*, 1981), and *P. hamadryas* (Kummer, 1968), although these events may not occur in a fixed order (Peng *et al.*, 1973; Rowell, 1977a). Sexual behavior has been noted in stump-tailed macaques long before puberty occurred (Trollope and Blurton Jones, 1975). Studies focusing on the onset of perineal

TABLE III

MENSTRUAL CYCLICITY IN PUBESCENT RHESUS MACAQUES AT OREGON (O) AND WISCONSIN (W) REGIONAL PRIMATE RESEARCH CENTERS[a]

Intermenstrual interval	Number of animals		Length of intermenstrual interval in days (mean ± SEM)[b]		% of total cycles in each category (days)		
	O	W	O	W	<24	24–34	>34
1st	22	26	93.6 ± 23.3	96.0 ± 14.0	14	8	77
2nd	22	26	55.7 ± 8.8	55.1 ± 6.9	12	19	69
3rd	22	26	62.1 ± 21.5	58.2 ± 8.6	21	25	54
4th	22	26	28.5 ± 1.9	50.7 ± 6.0	17	46	37
5th	22	26	48.7 ± 9.5	38.4 ± 5.4	23	42	35
6th	22	25	61.7 ± 13.6	37.3 ± 3.8	8	47	45
7th	22	25	43.4 ± 7.8	39.7 ± 5.0	11	47	42
8th	21	25	30.1 ± 2.9	63.2 ± 11.3	15	47	38
9th	21	22	56.4 ± 11.7	45.0 ± 8.9	14	42	44
10th	21	21	35.8 ± 2.9	32.9 ± 3.5	17	50	33
11th	21	19	40.8 ± 8.9	42.7 ± 10.0	12	58	30
12th	18	19	34.3 ± 4.6	42.5 ± 6.4	11	54	35

[a]From Resko *et al.* (1982).

[b]The first day of menstruation was designated Day 1 of the cycle.

tumescence as the first indicator of menarche in the pig-tailed macaque have shown that more than 75% of all females evaluated greater than 3 years of age were either pregnant or showed perineal swelling (Erwin and Erwin, 1976). In contrast, of those females between the ages of 2 and 3, 13–25% indicated either pregnancy or perineal swelling. There were no signs of tumescence in animals less than 2 years old.

Reports of captive female orangutans indicate that the onset of menarche is at about 7 years of age (Graham, 1988). Galdikas (1981) estimated first parturition at roughly 12 years with a 1 year cyclicity prior to conception. Harcourt *et al.* (1980) observed the first indications of sexual swellings in *Gorilla gorilla* at 7 years 9 months, with a duration of adolescent sterility of 10.5–18.5 months.

Some studies have shown a significant difference in the timing of the initiation of menarche. Van Wagenen and Catchpole (1956) reported that out of a group of 20 rhesus females, 10 matured "early" (1 year 8 months ± 43 days) whereas some matured "late" (2 years 5 months ± 59 days). The early menarche group appeared to gain weight more rapidly during the first year and showed an adolescent growth spurt earlier than the late-maturing group. Several other studies have suggested that weight gain may be related to the initiation of puberty, but this area still remains controversial (see Section II,A,2,c). Of interest is a report by Drickamer (1974) who noted that early maturing females were usually the daughters of high-ranking females.

Coe *et al.* (1979) reported that chimpanzees (*P. troglodytes*) raised in captivity tend to reach menarche earlier than in the wild, which has been noted in other species (Nieuwenhuijsen *et al.*, 1987). There have also been indications that the quality of rearing conditions may affect when onset occurs (Nadler *et al.*, 1987). Relocation of a natural troop of Japanese macaques (*M. fuscata*) from Arashyiama, Kyoto, Japan to south Texas resulted in a delay of sexual maturation by 1 to 2 years (Wolfe, 1979). These data and reports by others suggest that a variety of environmental factors, including stress, dietary changes, photoperiod, and environmental cues, may have a significant impact on the timing of puberty. Of interest are reports of decreased menarcheal age with increasing generations. The age of menarche for laboratory-born *M. arctoides* females steadily decreased throughout the period of captivity (Trollope and Blurton Jones, 1975); these results are similar to observations by van Wagenen (1972) in the rhesus monkey. Honjo *et al.* (1984) noted similar findings when comparing F_1 and F_2 generations of long-tailed macaques (*M. fascicularis*).

b. Hormonal Factors. In the female macaque, basal levels of luteinizing hormone (LH) are low during the prepubertal period, increase during early puberty (~20–30 months with circadian fluctuations), and begin to reach maximal amplitudes during the period of adolescent sterility (Terasawa *et al.*, 1983). Changes in LH secretory patterns do not appear to be the result of ovarian maturation since similar findings have been reported in ovariectomized females (Terasawa *et al.*, 1983; Terasawa, 1985; Terasawa *et al.*, 1984). Follicle-stimulating hormone (FSH) begins to increase at roughly 22 months, with maximal levels achieved at about 40 months. The FSH rise precedes LH and estradiol by about 5 months, and menarche by 6 to 8 months. In the rhesus, increased circulating estradiol occurs between 2.5 and 3 years, which is associated with nipple growth and an increase in perineal swelling and coloration (Terasawa *et al.*, 1983; Wilson *et al.*, 1984). Progesterone remains relatively low until the first ovulation. Foster (1977) hormonally evaluated the first 10 menstrual cycles of 10 sexually maturing indoor-housed macaques and found that the period of adolescent sterility may have been derived from a lack of ovulation (no LH surge observed, serum progesterone $\leq$1 ng/ml) in addition to short luteal phases (9 ± 7 versus 16 ± 0.2 days).

In the female stump-tailed macaque, no clear indication of reproductive maturity was observed except when monitoring serum progesterone concentrations (Nieuwenhuijsen *et al.*, 1987). Ovulation was noted when progesterone levels were $\geq$1.5 ng/ml.

Longitudinal studies of serum concentrations of the gonadotropins (LH, FSH) and sex steroids at 1- to 2-week intervals in juvenile chimpanzees indicate that levels remain low and stable in males, but that FSH is elevated randomly in conjunction with fluctuating concentrations of progesterone, estradiol, 17-hydroxy-progesterone, and androstenedione in females (Winter *et al.*, 1980). An association has been suggested between adrenarche and puberty onset in both the baboon and chimpanzee (Castracane *et al.*, 1981). However, further studies have shown that the two processes are dissociated and that there is no direct link between adrenal function and the process of sexual maturation (Nadler *et al.*, 1987).

Experimentally, normal ovulatory menstrual cycles have been induced by the pulsatile infusion of gonadotropin-releasing hormone (GnRH) in prepubertal female rhesus monkeys (Wildt *et al.*, 1980). Once infusion was discontinued, the animals reverted to the immature state. Conclusions drawn from these experiments were that (1) neither the adenohypophysis nor the ovary limit the initiation of puberty, and (2) this process is dependent on maturation of the neuroendocrine system which directs pulsatile secretion of GnRH from the hypothalamus.

c. Body Weight. The relationship between puberty and growth has been studied under several conditions. It has been suggested that a critical weight must be achieved prior to the initiation of menarche, although this hypothesis remains controversial. Schwartz *et al.* (1985) reported that for nine female outdoor-housed *M. mulatta*, four exhibited first ovulations (based on behavioral and endocrine parameters) during the fall and winter months (31.8 ± 0.4 months). Five of nine ovulated 1 year later at 41.8 ± 0.3 months, which was considered typical for the colony. These data support the contention that attainment of a critical body weight does not dictate the occurrence of first ovulation.

Using sex skin swelling as a marker, Erwin and Erwin (1976) found that out of 42 3-year-old females, 36 exhibited turgescence. Of the 6 nonturgescent females, 5 ranked lower in weight than any of the other females in the group. Only 7 of the 46 2 year olds evaluated exhibited swellings, 4 of which ranked highest in weight for their age, 1 ranked second, and 2 ranked third. These data appear to support the critical weight hypothesis, although weight alone does not explain the outcome for the turgescent 2 year olds whose weights were 0.25 kg greater than nonturgescent 3 year olds. In addition, a considerable overlap in weight was observed between the 2- and 3-year-old groups.

Reports by Terasawa *et al.* (1983) indicate that the highest growth rates in *M. mulatta* occur between 18 and 31 months (peripubertal) and between 38 and 47 months, which is prior to the first ovulation. The age of menarche for the colony studied was 30.7 ± 1.2 months with first ovulations noted 48.1 ± 2.2 months. However, it was also observed that the growth curves for body weight in neonatally ovariectomized females were almost identical to intact animals, which indicates that growth will occur regardless of the presence of ovaries and steroidogenesis. Further, body weights of macaques with hypothalamic lesions were significantly less than controls, indicating that the growth spurt prior to menarche and first ovulation was probably coincidental.

d. SEASON. It is generally accepted that the rhesus monkey housed outdoors is a seasonal breeder, with births normally occurring during the spring and early summer months (March through June). However, it has been suggested that season may also influence the timing of sexual maturation under these conditions. First ovulations, as indicated by hormonal and behavioral change, have been evaluated by Wilson *et al.* (1984) in rhesus macaques during the fall months. All females indicated signs of menstrual cyclicity by 30.6 ± 0.8 months. Significant elevations in pituitary and ovarian hormones were not observed until at least 41 months of age during the transition from summer to fall. The authors concluded that season may have influenced the timing of sexual maturation for outdoor-housed macaques since first ovulations were restricted to the fall and winter months.

Menstrual records from female rhesus macaques born at the Wisconsin Regional Primate Center (1970–1976; $N = 173$) indicated that those animals born between October and March were more likely to reach the age of menarche earlier than animals born between April and September (Terasawa *et al.*, 1983). In addition, first ovulations for 80% of the females studied occurred during October through January, which was attributed to the increase in estrogens noted around the winter solstice. In contrast, studies with (1) *M. mulatta* have not shown a relationship between seasonal factors and menarche onset or interval length (Resko *et al.*, 1982), and (2) stump-tailed macaques with hormonal evaluations and body weight measurements have not shown correlations with seasonality (Nieuwenhuijsen *et al.*, 1987). These observations have led to the conclusion that species may differ significantly in puberty initiation and that this variation may be due, in part, to environmental factors (Erwin and Erwin, 1976). It has also been proposed that counting the number of breeding seasons which have elapsed since birth may be more relevant than measures of absolute age in months.

e. NUTRITION. Nutritional and metabolic status have been implicated in determining the timing of normal puberty onset (Cameron *et al.*, 1985a,b; Schwartz *et al.*, 1988). Numerous reports have supported the hypothesis that reproductive dysfunction may occur under poor nutritional conditions. A link between circulating insulin levels and amino acids (i.e., tryptophan and tyrosine) and neurotransmitter synthesis suggests that these factors can have a significant impact on biochemical events within the brain, particularly in relation to GnRH neuronal activity (Cameron *et al.*, 1985a). The effect of a high-fat diet on sexual maturation in outdoor-housed rhesus macaques has been studied (Schwartz *et al.*, 1988). Results indicated an earlier onset of perineal swellings and menarche despite lower body weights [80% with first ovulations which is 40% earlier than controls (31–32 months of age)]. The authors concluded that high-fat diets may influence the rate of sexual maturation through metabolic factors which may have an impact on the developing neuroendocrine system.

f. TIME OF FIRST CONCEPTION. Several observations have been reported regarding the occurrence of first conceptions in relation to the initiation of menarche. Free-ranging rhesus monkeys at La Parguera, Puerto Rico, delivered their first infants at a mean age of 4 years (Drickamer, 1974); 12 of 56 (21%) delivered at 3 years of age, 33 of 56 (59%) at 5 years, and 1 of 56 (2%) at 7 years of age. The social rank of the individuals mother had a significant effect on the age of the first birth ($p<0.05$). Young females with high- and middle-ranking mothers delivered earlier (3.8–3.9 years) when compared to the offspring of the lower-ranking females (4.4 years).

Long-tailed macaques (F_1 generation) showed an average first delivery at 73 ± 20 months ($N=43$) versus F_2 generation females with a mean age of 63 ± 11 months of age ($N=6$) (Honjo *et al.*, 1984). Studies with *M. talapoin* and *E. patas* indicate conceptions at 4.5 and 2.5 years, respectively (Rowell, 1977a,b). Winkler *et al.* (1984) reported that *P. entellus* females will usually reach menarche at approximately 28.8 months with first conceptions at roughly 34.2 months. In the baboon, the age of first conception is usually between 3 and 4 years of age (Castracane *et al.*, 1981). Data for patas monkeys (Rowell, 1977a) are similar to the vervet (Bramblett *et al.*, 1975), namely the majority of females will have first conceptions between 2.5 and 3 years.

Chimpanzees in the wild show first conceptions at 8.2–10.7 years (Coe *et al.*, 1979) with 13 years reported as the mean age for first births (Harcourt *et al.*, 1980). This is in contrast to females in captivity (first births at 10–11 years), which correlates with the observation that chimps born and raised indoors

reach menarche earlier than those outdoors. Although data on the initiation of menarche were not collected, the age of first parturition in the gorilla was documented during the 10th year of life (Harcourt *et al.*, 1980).

B. Male

The most defined characteristics of this period of development in the male include both physical and endocrinological events, which are both closely associated. Body weight changes, testicular size and position, the presence of an ejaculate, elevations in testosterone, and conception are all interlinked with the attainment of sexual maturity.

1. Prosimians and New World Species

The endocrinology of puberty in the prosimians has not been extensively studied and, as a result, knowledge is generally based on the first matings. In the slow loris, sexual maturity is reached by 17 months of age; a pregnancy has been sired by a male of similar age.

In seasonal breeders, the onset of puberty does not occur in a slow progressive manner but rather emerges in a sudden fashion and resembles the adult annual reproductive cycle (Coe *et al.*, 1985). Prior to puberty, testosterone levels average 3 ng/ml; for adult males during the breeding season, testosterone surges between 7 and 40 ng/ml, which is sustained for the next 2 to 4 months. Squirrel monkeys, like marmosets, do not become sexually mature until about 18 months of age, although the latter are capable of copulating by 14 months.

In the tamarin, the testes are within the abdominal cavity at birth, descend to the inguinal canal at 2 to 3 months, and are found within the scrotum by 8 to 11 months. At 14 months of age, testicular changes such as tubular dilatation and an increase in tubular diameter occur. Mature sperm have not been documented until 16 months of age (Hampton and Taylor, 1971). In the owl monkey, testosterone is first detected at 211–337 days of age (Dixson, 1983). Males reach adult body weights (0.8–1 kg) between 370 and 520 days; no obvious adolescent growth spurt has been observed. In this species, a useful external indicator of puberty is provided by the growth of the subcaudal scent-marking gland. This structure is not present in juvenile males, but between 282 and 370 days the hairs in this region begin to stiffen and turn a brownish color. A fully mature gland is evident between 336 and 442 days of age; the growth and development of this gland correlates with increasing levels of testosterone during puberty.

2. Old World Species and the Great Apes

a. Testicular Changes and Spermatogenesis. One of the most obvious indications of male puberty is the onset of testicular growth. This closely correlates with development of spermatozoa and the presence of an ejaculate. Histological studies by van Wagenen and Simpson (1954) and evaluations of levels of plasma testosterone (Resko, 1967) indicate that the maturation and functional capacity of the testis occurs at 3 to 4 years in the rhesus male. This encompasses a prolonged period of slow development which is followed by a rapid succession of events associated with the differentiation of Leydig cells, an increase in Sertoli cell numbers, and the formation of spermatozoa. The Leydig cells of the testis, which produce testosterone, are very sparse or absent in prepubertal rhesus monkeys (Fouquet *et al.*, 1984; van Wagenen and Simpson, 1954), and there is minimal testosterone secretion during this period of development (Bercu *et al.*, 1983; Fouquet *et al.*, 1984; Martin *et al.*, 1977; Meusy-Dessolle and Dang, 1985; Winter and Faiman, 1972). Although the temporal relationship among testicular growth acceleration, Leydig cell development, and the increase in secretion of testosterone during early puberty still requires precise definition, it is probable that Leydig cell development and a rise in androgen levels in the testes precede both testicular growth acceleration and an increase in the circulating levels of sex steroids.

van Wagenen and Simpson (1954) noted the earliest appearance of spermatozoa for the rhesus at 2 years 11 months, with the latest observation at 3 years 5 months. For other species, information on the age at which spermatogenesis is initiated is generally lacking. In addition, few attempts have been made to correlate the morphology of the seminiferous tubules with the initiation of Leydig cell function. In both the rhesus and long-tailed macaque, well-defined morphological alterations in the seminiferous cords and tubules occur during prepubertal development in contrast to the relatively quiescent state of testicular steroidogenesis. The early pubertal acceleration of testicular growth is due mainly to an increase in both the diameter and tortuosity of the tubules which is associated with a proliferation of spermatocytes and the appearance of the definitive Sertoli cells.

The onset of spermatogenesis for *M. mulatta* precedes changes in perineal skin color (from blue or gray to red; 4.6 ± 0.7 years) (Vandenbergh, 1965). The mean age for testicular descent in this species is 3.3 ± 0.7 years (range of 2.3 to 4.3 years), which positively correlates with the beginning and peak of the adolescent spurt in linear growth. This is similar to observations by Goy *et al.* (1982) (3 years 1.3 months and 4 years 0.8 months), which is in direct opposition to the chimpanzee, where neither testicular descent nor perineal color changes are noted (Graham, 1988). Males in captivity reach puberty at 7 years 2 months ± 9 months with a body weight of 27.8 ± 5.8 kg. An increase in testicular size and testosterone begins at about 6 years, which precedes an increase in body weight gain (6 to 8 years) (Copeland *et al.*, 1985). This also contrasts reports on *M. radiata* (Glick, 1979) and *Papio* species (Copeland *et al.*, 1981) where a correlation has been shown between testes size and weight gain.

In *M. fascicularis*, prepubertal testicular weight has been noted to approximately double each year from 70 to 540 mg at

3 years of age (Kluin *et al.*, 1983). A further 25-fold increase occurs with puberty and adulthood, which is similar to observations for the rhesus (van Wagenen and Simpson, 1954). Dang and Meusy-Dessolle (1984) noted the appearance of spermatocytes at 3 to 4 years of age with a body weight of 3.2 ± 0.2 kg. Full spermatogenesis was achieved by 3 years 8 months to 4 years 4 months at a body weight of 3.5–3.8 kg, which is similar to the baboon (Castracane *et al.*, 1986). Steiner *et al.* (1983) studied 80 long-tailed macaques from 2 weeks of age to 8 years and divided the age groups as: neonates were classified as <3.5 months of age, infants as 3.5–8 months with a testicular volume of <0.3 cm^3, juveniles as 8–36 months with a testicular volume of 0.3–3.0 cm^3, peripubertals as 36–52 months with a testicular volume of 3–20 cm^3, and adults as >52 months with a testicular volume of >20 cm^3. An increase in testicular size was relatively stable until about 3 years when a dramatic increase was noted.

Completion of testicular descent for this species has been documented to occur at roughly 4 years of age (Steiner *et al.*, 1983). During the neonatal period, the testes were noted within the inguinal canals near the scrotal border. For older infants and juveniles, the testes were found high in the inguinal canal along the abdominal wall. At approximately 2 years of age they moved down the inguinal canal, and by 2.5 years were observed close to the scrotal border. With the onset of puberty (~44 months), the testes were completely descended into the scrotum. These events correlated significantly with changes in circulating sex steroid hormones, particularly testosterone.

Although generally macaques produce viable sperm at about 3.5 years, they are not considered socially mature nor do they contribute significantly to breeding until 2 to 3 years later (Honjo *et al.*, 1984; Rowell, 1977a). It should be noted, however, that a pregnancy has been sired by a long-tailed macaque male at 3.5 years of age (Honjo *et al.*, 1984).

Little has been documented regarding puberty in the larger species. Dixson *et al.* (1982) observed three male orangutans and noted that the process of spermatogenesis was complete at 6.4 to 7.7 years (0.05–0.30 ml with 60–90 million sperm/ml), although secondary sexual characteristics were lacking and weights were half of the expected adult size. Males have sired infants in captivity as young as 8 years of age; however, young males in the wild have not been observed to be sexually active until 10 years of age (Graham, 1988). Although males may become sexually mature at 7 to 10 years, the development of secondary sexual characteristics may be delayed for another 3 to 7 years (Graham, 1988; Kingsley, 1982).

b. HORMONAL FACTORS. The primary androgen formed by the testis is testosterone, which is responsible for the normal development of male structures. It may also be responsible for programming regions in the central nervous system (CNS) which regulate testicular function and male behavior. While normal differentiation requires the presence of testosterone, the development of the prostate and external genitalia requires dihydrotestosterone (DHT) for appropriate development (see Wilson, 1985, for review).

Initiation of the daytime rise of circulating testosterone at puberty has been observed to occur at 2.5–4 years in the rhesus and long-tailed macaque, African green monkey, sooty mangabey, and the baboon (Brady *et al.*, 1985; Mann *et al.*, 1989; Meusy-Dessolle and Dang, 1985; Plant, 1985) and between 7 and 8 years in the chimpanzee (Martin *et al.*, 1977). In the rhesus monkey the initial activation of testosterone secretion by the testis occurs nocturnally as early as 2 to 3 years of age, which is several months before the onset of the pubertal rise in daytime concentrations. The transition from a prepubertal to an adult pattern of testosterone secretion occurs relatively quickly; by approximately 3.5 years mean levels of androgens characteristic of adult males may be observed. Plasma LH in both rhesus and long-tailed macaques are slightly elevated during the first 6 months of age, then decline to very low levels for the next 2 to 3 years (Plant, 1988; Steiner and Bremner, 1981). FSH shows a similar pattern, and plasma prolactin begins to exhibit fluctuations after 1 year (Plant, 1985). Testosterone mimics LH and FSH postnatally; levels are high in the neonate, decline during late infancy, and reach baseline values during the juvenile period.

Hormonal patterns for a variety of androgens have been documented from birth to 6 years of age in *M. fascicularis* (Meusy-Dessolle and Dang, 1985). During the neonatal period (birth to 3 or 4 months of age), monthly mean levels are between 1.6 and 3.2 ng/ml; during infancy (3 to 4 months up to 29 months), very low levels (0.58 μl/ml) are noted; during the prepubertal phase (up to 43 months), circulating testosterone oscillates around a mean of 1.03 ng/ml with large individual variability, and a pubertal phase (mean of 43 months) occurs when testosterone concentrations rise to a mean of approximately 7 ng/ml, which coincides with the establishment of spermatogenesis. Testosterone continues to rise until adult levels of roughly 20 ng/ml are achieved. DHT concentrations parallel testosterone, with high concentrations noted during the first 4 months of age (monthly mean of 0.94–1.80 ng/ml), which plateau at ~0.25 ng/ml at ~29 months of age.

In the chimpanzee, measurements of serum testosterone indicate that juveniles (1 to 6 years of age) display mean values of 0.13 ng/ml (range of 0.04–0.59 ng/ml), adolescents (7 to 10 years) show a mean of 1.78 ng/ml (range of 0.15–2.38 ng/ml), and adults (≥11 years of age) show a mean of 3.97 ng/ml (range of 0.92–6.80 ng/ml) (Martin *et al.*, 1977). The earliest conception recorded with Yerkes' males occurred at 9 years 2 months, which was roughly 2 years following the initial rise in serum testosterone (7 years). A gradual increase in the level of circulating hormones has been reported in male orangutans (Graham, 1988); testosterone levels of 1606 and 2367 ng/ml have been observed in an 11-year-old subadult and a 14-year-old adult, respectively. Actively breeding and nonbreeding males with fully developed secondary sexual characteristics such as flanges have estrogen levels roughly double when compared to

nonflanged adults. Testosterone in flanged males is either equal to or twice as great as levels for nonflanged males.

c. BODY WEIGHT. In the rhesus, the adolescent growth spurt is closely related to the development of secondary sexual characteristics and occurs at roughly 2.5 years (van Wagenen and Catchpole, 1956). This is contrary to observations in females of this species, where puberty precedes the growth spurt (~1.5 years) and shows little relation to it. Long-tailed macaques show a relatively linear rate of weight gain up through 3 years of age when the variability suddenly increases which reflects the pubertal growth spurt (36–52 months) (Steiner *et al.*, 1983). This correlates with testicular size which remains stable until approximately 3 years.

For stump-tailed monkeys, testicular growth coincides with body weight growth and the attainment of adult testosterone levels (Nieuwenhuijsen *et al.*, 1987). However, some males show a period of a relatively low or negative body weight increase which coincides with little testis growth.

Male baboons show a distinct adolescent growth spurt at 3 to 4 years (Castracane *et al.*, 1986; Glassman *et al.*, 1984) which is similar to macaques (van Wagenen and Catchpole, 1956) and chimpanzees (Graham, 1988). During this period of development, a greater variation in body weight may be observed in males when compared to females which is not evident by approximately 6 years of age. Chimpanzees show a growth spurt at 9.5 years (peak at ~11 years) which is a relatively late event during adolescence considering that they reach puberty at 7 to 8 years (Martin *et al.*, 1977; Smith *et al.*, 1975). A significant size difference between the male and female orangutan begins at puberty; sex:weight ratios may be as high as 1:2 (females 35–50 kg, males 45–100 kg) (Eckhardt, 1975; Graham, 1988). For *G. gorilla*, an adolescent growth spurt has been noted to occur between 6 and 7 years of age (Dixson, 1981).

d. SEASON. Rhesus males show seasonal increases in sexual behavior during the second and third year prior to the rise in plasma testosterone. This species has shown a rise in both LH and testosterone during the third year of life with rapid decreases in the fall months which coincides with the breeding season (Mann *et al.*, 1989). This is in contrast to the long-tailed macaque where no significant season-related differences have been noted (Dang and Meusy-Dessolle, 1984; Kluin *et al.*, 1983).

For *M. fuscata*, seasonal changes in reproductive phenomena include activity of the testis, which repeats on an annual cycle with maximal activity noted during the mating season, regression during the birth season, and redevelopment toward the following mating season (Nigi *et al.*, 1980). These seasonal changes were first noted in 4- to 6-year-old males during the process of sexual maturation. Similar to changes in testis size, noticeable changes in plasma testosterone concentrations according to age were observed during this same period of development, which is similar to adults. The process of maturation in this species occurred over a 2-year period, with full maturity achieved at ⩾6.5 years of age. It was concluded that based on testis size, plasma testosterone, and seminiferous epithelium, gonadal activity developed rapidly during a short period of time and, although spermatogenesis started during the mating season at 4 years of age, full sexual maturation was attained 2 years later.

In a study of adult, adolescent, and juvenile male bonnet macaques (average age of 8.0, 3.4, and 1.9 years, respectively), strong correlations between testis size and age, and testis size and body weight were observed over two breeding seasons (Glick, 1979). The adolescent males displayed smaller testes during each season when compared to adults, and testicular size during the mating season fell within the adult range during the nonbreeding season. The testes of the adolescents and juveniles did not differ significantly in size during the nonbreeding season but there was a significant increase in the testes of the adolescents with the onset of the mating season. Testicular growth occurred continuously throughout maturation and accelerated during adolescence. These seasonal observations were first noted in males at 3.4 years of age. The cyclical changes in the testes and testosterone paralleled the breeding season and were a predictable characteristic of sexual maturity for the species. Body weights were, however, not subject to seasonal change.

e. NUTRITION. Several nutritional and metabolic factors have been implicated in the initiation of puberty (see Section II,A,2,e). Although body size, body fat, and metabolic rate have all been associated with normal reproductive function, the mechanism by which they interact remains to be elucidated. It has been noted that dietary restriction can result in a decline in both body weight and circulating gonadotropins when food intake is reduced in castrated males (Dubey *et al.*, 1986). The observed decrease in FSH and LH was, however, restored by an infusion of GnRH. It has been suggested that insulin or amino acids could provide the link between nutritional status and reproductive function by influencing the synthesis of neurotransmitters critical for maintaining GnRH secretion (Steiner *et al.*, 1983). Studies with *M. fascicularis* have shown that chronic administration of amino acids and glucose stimulate adult-like LH/FSH presumably through the release of GnRH (Cameron *et al.*, 1985a,b). It was concluded that blood-borne metabolic cues which specifically sustain elevation of insulin (induced by glucose) can stimulate the activity of GnRH-secreting cells and that these factors may be responsible for mediating maturational events within the brain.

Precocious puberty has been induced in the rhesus male by an intravenous injection of *N*-methyl-D-aspartate, an analog of the excitatory neurotransmitter aspartate (Plant *et al.*, 1989). Prolonged stimulation at 16–30 weeks of age resulted in the onset of puberty with the initiation of spermatogenesis. Somatic growth was also markedly increased after treatment. These findings provide evidence that the network of hypothalamic GnRH neurons, the pituitary, and gonads is a nonlimiting component

TABLE IV
ESTROUS CYCLES IN PROSIMIANS AND NEW WORLD SPECIES

Species	Estrous cycle (days)	Estrous length (days)
Prosimians		
G. crassicaudatus	39	—
G. moholi	29–39	5–7
L. catta	39	4–5
L. fulvus	30	—
L. macaco	33	3.5
L. mongoz	29–48	—
L. tardigradus	29–40	2
M. murinus	38–55	3
N. coucang	29–45	1
Tarsius	24	1
V. variegata	25	—
New World species		
A. trivirgatus	15–16	—
Alouatta	16	—
Ateles	24–27	—
Callithrix	27–30	—
Cebus[a]	(18–23)[a]	—
S. sciureus	7–12	?
Saquinus	15–22	—

[a]Vaginal bleeding indicative of menstrual cyclicity (see text).

of the control system that governs the onset of puberty in this species. Readers are referred to select reviews on this topic for more complete information.

III. ESTROUS AND MENSTRUAL CYCLES

A. Prosimians and New World Species

1. Estrous Cycles

Menstruation does not occur in prosimian or New World primates, indicating true estrous cycles for these species. Mean cycle lengths for a variety of prosimian and New World monkeys are listed in Table IV. In the prosimians, cyclicity can be monitored by the detection of the dilatation or constriction of the vaginal orifice and by obtaining vaginal smears which are useful for identifying cornified squamous cell. In addition, the cycles of both prosimians and New World species can be monitored by an assay of serum or urinary hormones or changes in behavior (see Chapter 14). Estrous cycle lengths vary significantly among the *Galago* species. Most reports indicate cycle lengths of 40 days for *G. crassicaudatus* (Eaton *et al.*, 1973; Hendrickx and Newman, 1978), but mean cycle lengths of 50.3 days have also been reported (Valerio *et al.*, 1972).

a. HORMONAL INTERACTIONS. Plasma steroid concentrations have been reported for only a few of the prosimians. A mean cycle length of 44 days, with a luteal phase of roughly 24 days, has been observed for *G. crassicaudatus* by Eaton *et al.* (1973). At the midcycle peak and for 5 days after, mean estrogen concentrations of 514 ng/ml were detected. The peak correlated with histologic changes in the vaginal smear and with the period of sexual receptivity. Progesterone levels remained high throughout the luteal phase, with a mean of 8.6 ng/ml.

For the ring-tailed and ruffed lemur, luteal phases of 24–25 days were determined (Robinson and Goy, 1986), whereas a slightly longer luteal phase (28 days) was reported for *L. macaco* (Bogart *et al.*, 1977), with peak progesterone concentrations between 12 and 48 ng/ml 5 days after estrus. Van Horn and Resko (1977) found progesterone levels of 92.5 ng/ml at 26 days after the estradiol peak; peaks of 300–450 pg/ml were observed during estrus whereas much lower levels were reported during proestrus, with basal concentrations of 10–20 pg/ml. Sexual receptivity coincided with the day after the estrogen peak and lasted about 24 hr.

Traditional mating behavior (as a sign of estrus) is difficult to detect in squirrel monkeys; therefore, vaginal cytology is more commonly used. The cycle length for this species ranges from 7 to 12 days, with most cycles between 8 and 10 days. A white discharge consisting of desquamated vaginal cells can be observed on occasion, although this phenomenon is quite variable in both its incidence and time of occurrence. It is uncertain if all cycles are ovulatory in the squirrel monkey since short cycles have been reported to be associated with a higher incidence of anovulatory cycles (Dukelow, 1983, 1985). The squirrel monkey, like other New World primates, exhibits very high levels of steroid hormones (Wolf *et al.*, 1977; Diamond *et al.*, 1984) and it appears that these elevated levels are compensated for by reduced numbers of hormone receptors (Chrousos *et al.*, 1984). During estrus, pregnanediol excretion increases abruptly on the sixth day of the cycle indicating ovulation. Serum progestins reach a peak concentration of nearly 400 ng/ml 3 to 4 days after the estradiol peak with maximum estradiol values approximately 500 pg/ml. Serum estradiol and LH levels increase significantly on Day four of the cycle, immediately prior to ovulation. The LH surge is of 1 to 2 days duration and is comparable in amplitude to other primates (Ghosh *et al.*, 1982; Yeoman *et al.*, 1988).

The estrous cycle of the common marmoset has been reported to be 28.6 ± 1.0 days (Hearn, 1983; Kholkute, 1984), with a follicular phase of 8.3 days and a luteal phase of 19.2 days. There are no externally obvious changes that occur in this species that can be used to predict ovulation (Hearn, 1983). Peripheral plasma levels of progesterone are ≤5 ng/ml prior to ovulation and nearly quadruple within 1 day postovulation. The rapid increase in progesterone is a reliable and useful means of timing ovulation in this species. In the saddle-back tamarin, cycle length averages 17.3 days compared to 15.2 days in *Saguinus oedipus*. Estradiol levels remain low as long as females are members of their natal families. This social suppression has also been noted in the marmoset (Abbott *et al.*, 1988). Pairing

with an adult male results in an immediate increase in estradiol levels which results in normal peak values.

The owl monkey does not show cyclical changes in either swelling or coloration of the external genitalia. Furthermore, vaginal cornification is of limited value in determining the stages of the cycle in this species. Accordingly, steroid measurements of urine or serum are necessary to evaluate changes with estrus. The average cycle length is 15–16 days for this nocturnal species. Estrogen concentrations rise sharply from Day 0 to a peak on Day five of the cycle, then decline to baseline concentrations by Day 13. Progesterone levels increase 24 hr after the estrogen rise, peak on Day 8, and decline to basal levels by Day 11. As with other New World primates, peak levels of sex steroids are extremely high, with estrone approaching 3.6 ng/ml and progesterone roughly 250 ng/ml.

The howler monkey experiences a regular estrous cycle averaging 16.3 days with obvious sex skin changes. The cebus monkey has a cycle duration ranging from 18 to 23 days; the follicular phase lasts 8.3 days and the luteal phase approximately 11.7 days. Plasma concentrations of estradiol range from 50 to 150 pg/ml during the first 5 days of the cycle with a rapid increase to a peak of 540 pg/ml between Days 7 to 10 which is roughly about 10 to 24 hr prior to ovulation. These concentrations remain stable until the onset of the next cycle. Progesterone levels remain low during the follicular phase, then increase within 12 hr of the estradiol peak which ranges from a low of 5 ng/ml during the follicular phase to maximum levels of 60–100 ng/ml during the midluteal phase. These levels are 8–12 times higher than those found in Old World monkeys. This New World species does show occasional tinges of blood in vaginal secretions and, although controversial, is considered to be true menstruation.

Steroid concentrations have also been reported for the estrus cycle of the greater galago (Eaton *et al.*, 1973), with mean estradiol levels at midcycle of 519 pg/ml. High circulating concentrations of estradiol remain at these concentrations for at least 5 days and correlate with changes in vaginal cytology. Progesterone concentrations are elevated during the luteal phase with a mean peak concentration of 8.6 ng/ml. Progesterone concentrations in the ring-tailed and ruffed lemurs are less than 2.0 ng/ml during the follicular phase whereas concentrations range from 12 to 48 ng/ml 5 days after estrus and 92.5 ng/ml 26 days after the estradiol peak (Bogart *et al.*, 1977; Shideler and Lindburg, 1982). Estradiol concentrations are usually less than 200 pg/ml in these species with baseline levels roughly 10–20 pg/ml. In the ring-tailed lemur, the luteal phase is proportionately long (25 days) compared to a 14-day follicular period. Progesterone levels reach 17 ng/ml 5 days after estrus and the average maximum is about 22 ng/ml, but with wide variation between individuals.

Unfortunately, the activities of the hypothalamic and hypophyseal (pituitary) hormones have not been extensively studied in the prosimians; therefore, one must extrapolate from findings in other primate species. The role of steroid hormones and the ovulatory cycle, however, has been reviewed (Dukelow *et al.*, 1986; Robinson and Goy, 1986).

In most primate species, oogenesis occurs prior to birth with cessation postnatally. An interesting exception is found in the loris where continuation of this process occurs into adult life (Anand Kumar, 1968; Butler and Juma, 1970).

b. SEASONALITY. Seasonal aspects of reproduction have been reviewed by Lindburg (1987). Varying times and lengths of breeding seasons occur in prosimians, with the shortest noted in the ring-tailed lemur (2 weeks). The dwarf and the mouse lemur have a breeding season between October and February with birth occurring from the end of August to November, although when transported to the northern hemisphere, the seasons are transposed. The lesser galago has two separate breeding and birth seasons, with offspring born in October and early November and again in late January to March. In contrast, the greater galago has a single birth season in November. Although strict seasonality occurs with these species in the wild, this is usually not maintained in captivity.

In the galago, the vaginal orifice is sealed for the large part of the estrous cycle. At the time of proestrus there is an increase in the swelling and coloration of the vulva and labial folds, and during full estrus, the labial folds and perineum become turgescent and a clear mucous secretion can be observed. In late estrus, detumescence occurs, the color of the labia wanes, and the vagina, while remaining open, diminishes its mucous secretion.

The slender loris in captivity does not show breeding seasonality since mating and conception are observed throughout the year. Estrus cycles occur in all months of the year in this species but appear with a greater frequency in the last 6 months of the calendar year. There appears to be strong evidence that slow loris' housed under controlled photoperiod have a restricted birth season (Izard *et al.*, 1988). In lemurs, the photoperiod control of estrous cycles has been well-documented (Van Horn, 1980) and most captive species breed during periods of decreasing daylight. In many lemur species in the northern hemisphere, male sexual activity begins in late October with estrus and mating occurring in late November or December. The mongoose lemur breeds slightly later during the months of December through March.

Most members of the lemur family are seasonally polyestrous, having as many as four estrous cycles annually. With the ruffed lemur, births occur in the northern hemisphere from late March through June and July, with a birth peak in April and May. In this species, three cycles occur annually and pregnancy can occur in any of these, although generally females conceive during the first cycle.

Courtship and mating behavior have been studied in a number of prosimians. In the lesser galago, male sexual interest remains at a constant level throughout the year. The male approaches the female to examine the genital organs which is usually followed by "urine marking" by the male, which is a general characteristic of the galago and loris. Urine marking

consists of the male obtaining a few drops of urine in a cupped hand, which is then wiped on the sole of the foot; females urine mark in the same way, but less frequently. The sexual significance of this act is unknown (Doyle *et al.*, 1967). At the time of estrus, urine marking by the male increases significantly. Courtship is always initiated by the male and, during estrus, the female will exhibit a white vaginal discharge which appears to excite the male. In the slender loris, mating behavior is characterized by several bouts of copulation of a few seconds to 2 min in length. Prior to copulation, the male and female suspend themselves under a branch with the female supporting the weight of both. In the slow loris, copulation can take as long as 20 min and occurs with the female hanging upside down from a horizontal branch. In the ring-tailed lemur a definite female sexual pattern is observed during mating which consists of initially stiff-legged posture with brief runs, followed by a receptive stance with the hind legs extended and the tail brought forward with its tip curled (Evans and Goy, 1968; Koyama, 1988; Shideler *et al.*, 1983).

The seasonality of reproductive response is well-established for the squirrel monkey. In the wild, births occur over a distinct 8- to 12-week period each year. If the animals are moved to the northern hemisphere there is a marked shift in the mating and birth seasons which has been attributed to the degree of precipitation. It has been suggested that the start of the dry season, regardless of hemisphere, triggers mating although the exact mechanism is not known. The seasonality effect is observed in both the female response and in ovulation induction regimens even under controlled conditions. This response has been attributed to alterations in the sensitivity of the hypothalamic–pituitary axis relative to FSH secretion (Kuehl and Dukelow, 1975), although photoperiod does not appear to play a controlling factor in these seasonal changes. Adaptation to captivity is important in this species and, while some animals will adapt by 6 months of captivity (Harrison and Dukelow, 1973), at least three breeding seasons are usually required for adaptation of the female. There are also changes in body weight on an annual pattern which occur in both males and females by the time the animals are 4 years of age. These are more conspicuous in some subspecies (i.e., Colombian males) than in others. Weight fluctuation in females is less evident than in males, but does occur (Kaplan *et al.*, 1981). The occurrence of behavioral estrus is also seasonal and independent of physical contact with males.

No seasonality is observed in marmosets or tamarins; in wild or seminatural conditions there is mating of a single dominant female and hormonal suppression of the others. Signs of estrous activity are difficult to identify in the female tamarin but scent marking does occur and often the behavior of the male is indicative of female cyclical changes (French *et al.*, 1984). These species migrate from group to group often, therefore, consistent membership within the group is unstable. Under conditions of controlled lighting and temperature, owl monkeys show no apparent annual birth peak or birth season although under wild conditions it is uncertain whether the owl monkey is a seasonal breeder considering their nocturnal nature. These species do not show consistent changes in the frequency of copulatory behavior, scent marking, or grooming when paired. Similarly, during mating, the males do not vary in the frequencies of anogenital inspections, mounting attempts, or mounts with pelvic thrusts, and male owl monkeys do not exhibit the body tremor associated with thrusting as noted with other primate species. There is also no evidence of seasonality in the mating of the cebus monkey.

TABLE V

OVARIAN (MENSTRUAL) CYCLES IN OLD WORLD MONKEYS AND GREAT APES[a]

Species	Cycle length (days)	Menstruation[b] (days)	Sex skin[c]
C. aethiops	30–33	1–2	–
C. patas	30–34	Rare	–
C. talapoin	33	2–6	+C
M. mulatta	26–30	4.6	+C
M. fascicularis	28–32	2–7	+
M. arctoides	28–29	—	?
M. cyclopis	29	3.3	+C
M. fuscata	26–28	3.5	+C
M. nemestrina	29–32	—	+C
M. radiata	25–36	10	+
M. silenus	40	2.5	+C
M. sinica	29	1–4	–
M. sylvana	27–33	3–4	+C
P. entellus	21–26	—	–
P. troglodytes	31–37	3	+C
P. pygmaeus	24–32 (30.5 mean)	—	–
G. gorilla	28	—	–

[a]Data from Hartman (1932); Butler (1974); Spies and Chappell (1984); Robinson and Goy (1986); and Hrdy and Whitten (1986).

[b]External signs, unless otherwise noted.

[c]–, absent; +, present; C, cyclic.

B. Old World Species and the Great Apes

1. Menstrual Cycles

Unlike estrus which occurs at the time of maximum follicular growth and estrogen production, menses occurs when there is little hormone secretion. In most species, the menstrual cycle lasts approximately 28–30 days, with ovulation occurring during Days 12 to 15 (Table V). Confusion can occur regarding the categorization of cyclicity as "ovarian" versus "menstrual," although virtually no distinction exists between the two. Ovarian cyclicity refers to three distinct events: follicular growth, ovulation, and formation of a corpus luteum (CL) (Goodman and Hodgen, 1983), whereas menstrual cyclicity infers the period of time between the onset of vaginal hemorrhage from one cycle to the next. Theoretically, species exhibiting the vaginal bleeding associated with menstruation possess uterine spiral

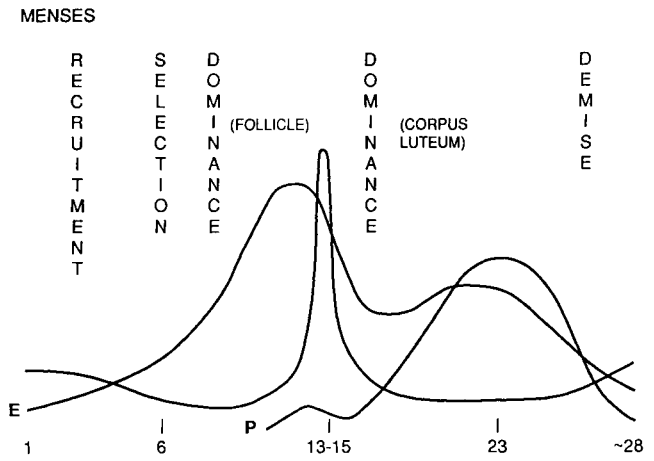

Fig. 1. Terms used to describe the sequence of principal ovarian events during follicular maturation and corpus luteum function are temporarily defined in the menstrual cycle. The curves depict idealized (stereotypical) patterns of the pituitary gonadotropins, estradiol (E), and progesterone (P) in the peripheral circulation. From Hodgen (1982). Reproduced with permission of the publisher, The American Fertility Society.

arteries which are capable of responding to hormonal withdrawal at the end of the luteal phase. In addition to the vaginal hemorrhage normally observed with cyclicity, other external signs, including alterations in sex skin color, sexual behavior, and/or perineal swelling, may provide opportunities for the detection of the stage of the cycle, depending on the species.

Most of the material covered in this section is related to the rhesus (*M. mulatta*) and/or long-tailed macaque (*M. fascicularis*), as the majority of studies centered around cyclicity have incorporated these two species. In many cases, both have been studied in combination since they are similar physiologically (Dukelow, 1983; Kerber and Reese, 1969; MacDonald, 1971). It should be noted, however, that while the rhesus remains a seasonal breeder (even when housed under controlled conditions), the long-tailed macaque appears to be reproductively unaffected by season (MacDonald, 1971). The basic features for all species discussed are similar; variation can be found regarding the duration of each component of the cycle and the hormone levels achieved.

a. Hormonal Interactions. Changes noted throughout the cycle are uniquely dependent on the hormonal milieu and the interaction of the hypothalamus/pituitary (gonadotropins), ovary (estrogens and progesterone), and the uterus. The first half of the cycle is characterized by the growth and development of several follicles within the ovary (follicular phase) and the development of the endometrial mucosa of the uterus (proliferative phase). The process by which the ovarian follicles are "chosen" for maturation out of the pool available ("reruitment" or "activation") is currently unknown (Fig. 1). In addition, the mechanism responsible for determining which will reach the final stages of maturity while the others become atretic ("selection") is even less well understood (DiZerega and Hodgen, 1981), although the dominant follicle (DF) is usually identifiable by Days 6 to 8 of the cycle (DiZerega and Hodgen, 1981; Goodman and Hodgen, 1983). Hormonal analyses, either by serum samples or urine collection, provide the most accurate means for identifying ovarian activity and the stage of the cycle (Czekala *et al.*, 1988; Monfort *et al.*, 1986, 1987; Knobil and Hotchkiss, 1988). Estradiol secreted by the developing follicles is characteristic during the follicular phase. With further maturation of the DF, increasing levels appear in both the peripheral circulation and as increasing estrogen conjugates in the urine (Czekala *et al.*, 1988; Hodgen, 1982; Monfort *et al.*, 1986, 1987). FSH is the primary control mechanism for the biosynthesis of estradiol by the ovary, with the principal source of estradiol production probably within the granulosa cells. In conjunction with FSH, LH is responsible for interacting with the theca cells. Both the granulosa and theca cells act in concert in the synthesis of estradiol ("two-cell theory"); steroid hormone production is a cooperative interaction between these two ovarian cell types.

Steroid hormone concentrations have been measured throughout the cycle for a variety of macaques which include the rhesus (Fig. 2), long-tailed, stump-tailed, Japanese, pig-tailed, and bonnet macaque (see Robinson and Goy, 1986, for review). For the majority studied, the profiles appear similar, although some differences such as greater hormone concentrations or differences in the relationship of estradiol to the LH peak may be observed. During the early follicular phase, concentrations of estradiol for the rhesus are 50–100 pg/ml (Bosu *et al.*, 1972, 1973; Hess and Resko, 1973; Hopper and Tullner, 1967; Hotchkiss *et al.*, 1971; Weick *et al.*, 1973). An estradiol peak of 350 pg/ml will occur 9–15 hr prior to the LH peak and at approximately 36 hr prior to ovulation. The long-tailed and bonnet macaque are both similar, with 50–150 pg/ml estradiol and peak concentrations of 200–350 pg/ml for *M. fascilularis* and 75–125 pg/ml with a peak of roughly 200 pg/ml for *M. radiata* (Goodman *et al.*, 1977; Kholkute *et al.*, 1981; Lasley *et al.*, 1974; Parkin and Hendrickx, 1975; Saldarini *et al.*, 1972; Shaikh *et al.*, 1978). *M. fuscata* has secretory patterns similar to the rhesus, although the levels are somewhat higher, with midcycle peaks of ~500 pg/ml. In contrast, *M. nemestrina* has a longer follicular phase when compared to other macaques and, therefore, an extended period of estrogen production (Blakley *et al.*, 1981), with peak levels of roughly 450 pg/ml. Other characteristics unique to this species include a secondary rise in estradiol 6 to 8 days into the luteal phase, changes in sex skin swellings which follow the estradiol pattern (mean time from peak turgescence to ovulation = 6.3 ± 1.1 days), and a closer correlation of the estradiol and LH peaks. Baboons (*P. cynocephalus, P. anubis, P. hamadryas*, and *P. ursinus*) show estradiol concentrations of ~50 pg/ml in the early follicular phase, with peak concentrations of 250–350 pg/ml ~24 hr prior to the LH peak (Stevens *et al.*, 1970). No secondary rise occurs

during the luteal phase. A similar correlation as noted in pigtailed macaques regarding perineal sex skin changes is also seen in these species. Turgescence occurs during the initial elevations of estradiol, with maximum turgescence at the time of the peak or 3 to 4 days after the urinary estrogen peak; detumescence occurs during the luteal phase (see Chapter 14). Concentrations of estradiol in the chimpanzee are ≤ 50 pg/ml during the early follicular phase which increase to a peak of 120 or 350 pg/ml (Graham, 1981b; Reyes *et al.*, 1975). Values then decline with a secondary rise during the luteal phase of ~80 to 100–180 pg/ml, although the timing of the secondary rise may be highly variable. Detumescence appears to be closely related to ovulation, with the last day of maximum swelling defined as the day of ovulation.

A study by Nadler *et al.* (1984) in orangutans indicates early serum follicular estradiol levels of <100 pg/ml with midcycle peaks of 163–318 pg/ml; luteal phase levels were 56–316 pg/ml. Nadler *et al.* (1979) monitored female gorillas and found plasma estradiol concentrations of N <100 pg/ml during the follicular phase which sharply increased to peak levels of 200–500 pg/ml midcycle.

Although progesterone is barely detectable during the follicular phase, the formation of the CL postovulation (*luteal phase*) results in the secretion of high concentrations. The luteal phase of the ovary is accompanied by the *secretory phase* of the uterus; both are dominated by the high levels of progesterone produced by the CL. Generally speaking, the production of progesterone begins to decline shortly after Day 21 of the cycle, and the onset of menstrual flow begins approximately 3 days after plasma progesterone concentrations reach baseline levels. Estrogens are also produced in large quantities by the CL but the time course of plasma estrogen concentration in Old World monkeys is markedly attenuated whereas it approximates that of progesterone in the chimpanzee (Graham, 1981b; Reyes *et al.*, 1975), orangutan (Graham, 1981b; Nadler *et al.*, 1984), and gorilla (Graham, 1981b; Nadler *et al.*, 1983). The circulating levels of the gonadotropins during the luteal phase are lower than those observed during the follicular phase, but will rise slightly toward the end of the cycle (Knobil and Hotchkiss, 1988).

Typically, as seen in the rhesus, luteal phase concentrations of progesterone increase from <0.5 ng/ml to a maximum of 4–6 ng/ml on the 15th day of the cycle. These levels remain relatively constant for roughly 7 days and then decline to follicular phase levels prior to menses (Clarke *et al.*, 1978; Hodgen *et al.*, 1976; Hopper and Tullner, 1970; Niswender and Spies, 1973; Resko *et al.*, 1974; Stabenfeldt and Hendrickx, 1973; Weick *et al.*, 1973). For the orangutan, luteal phase progesterone ranges from 5.7 to 13.8 ng/ml (Nadler *et al.*, 1984). During this stage of the cycle, the development of new follicles is inhibited by the high concentrations of circulating progesterone. The functional lifespan of the CL is roughly 14 days, which accounts for the 28-day cycle normally observed in most species. When luteolysis occurs, new follicles are then recruited and a new cycle begins (see Fig. 1).

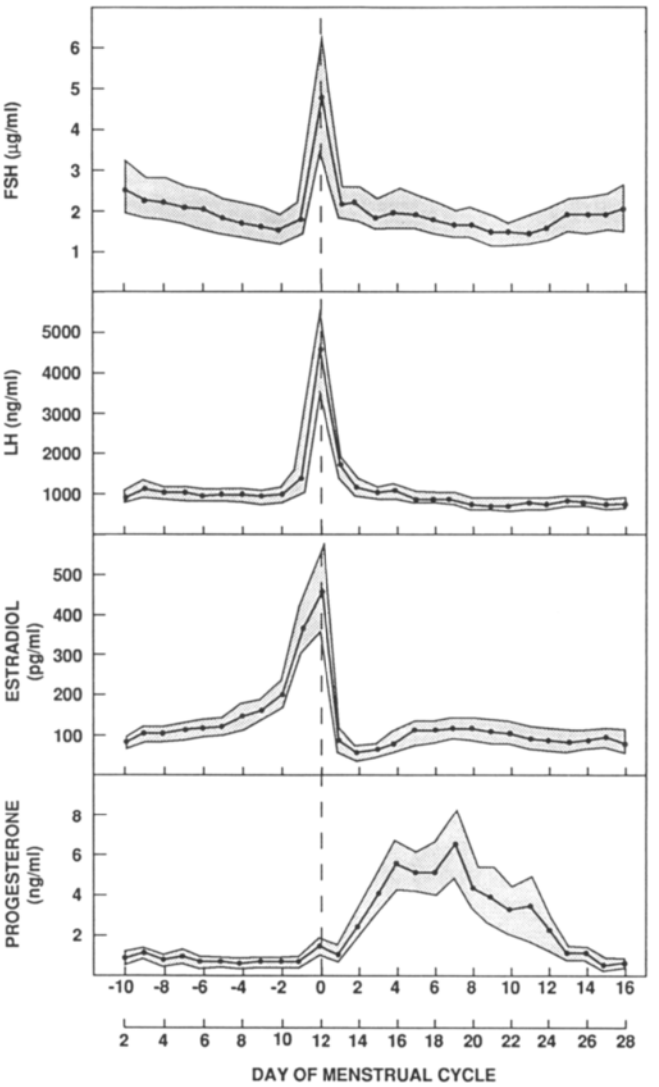

Fig. 2. Serum concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, and progesterone in 20 rhesus monkeys *(Macaca mulatta)* during ovulatory menstrual cycles. From Hodgen *et al.* (1976). Copyright © 1976 by The Endocrine Society.

b. Ovarian Changes/Timing of Cycle. Morphological changes in the ovary are directly related to the hormonal environment, as described earlier. The duration of the cycle and timing of the major changes within it are directly by the ovary and its interaction with the hypothalamus (Knobil and Hotchkiss, 1988). Development is dependent on the GnRH pulse generator which is located within the arcuate area of the mediobasal hypothalamus. The timing of these events is critical and discrete morphological changes must occur in a fixed sequence. Readers are referred to reviews regarding morphology associated with

follicular development (Dukelow, 1975; Jewett and Dukelow, 1972b; Koering, 1987).

The development of a tertiary (Graafian) follicle, its production of estradiol, the formation of a CL postovulation, and the secretion of progesterone are the critical events which dictate normal cyclicity. Located in the ovarian cortex, the primordial follicles closest to the medulla are usually the first to be stimulated (Koering, 1987). Antrum formation begins when the follicles reach 200–250 μm, as fluid-filled spaces develop within the granulosa cells. With further development, all but one will degenerate; the majority of developing follicles become atretic at 0.5–1.0 mm (Koering *et al.*, 1982). It should be noted that studies on follicular atresia have shown that biochemical changes precede morphological degenerative alterations by 24 hr (Hutz *et al.*, 1989). Detection of the DF occurs prior to Day 8 of the cycle in most nonhuman primates (Goodman and Hodgen, 1983; Koering *et al.*, 1982). Preovulatory follicular diameters may vary with the species; the rhesus and baboon usually develop follicles in the range of 5.0–6.5 mm. The initiation of the maturation process is dependent on FSH, and studies in the bonnet monkey have indicated that this requirement predominates the first 7 days of the 9 to 10 days required for follicular maturation (Ravindranath *et al.*, 1989). It was also noted that the mature follicle becomes independent of FSH ~48 hr prior to ovulation.

Major changes occur in the ovary immediately prior to ovulation which include alterations in follicular vasculature and formation of an ovulatory stigma (Dukelow *et al.*, 1986). A variety of theories have evolved regarding the mechanism by which ovulation occurs, including the role of prostaglandins (PGs). With collapse of the follicle, the wall of the ruptured DF becomes luteinized, and a change in the ovarian hormonal environment occurs. The induction of enzymes required for the synthesis of progesterone results in its high production. Estradiol is produced by the CL, although it will negatively feedback on the lutein cells to inhibit production of androstenedione, a precursor in the pathway to the production of estrogens.

Studies with the Japanese macaque have shown that luteinization may begin prior to ovulation (Nigi, 1978), which has also been suggested in studies with *M. fascicularis* (Jewett and Dukelow, 1971, 1972b). In the Japanese macaque, the formation of the CL progresses gradually during the first 40 hr postovulation. When pregnancy does not occur, the CL secretes both progesterone and estradiol for about 12 days before the decline of hormone production induces menstruation (Knobil and Hotchkiss, 1988; Spies and Chappel, 1984). The production of progesterone by the CL requires the presence of LH, although it has been shown that the maintenance of the functional capacity of the CL is less dependent on LH (Zeleznik and Hutchison, 1987). Results from other studies indicate that spontaneous luteal regression is not related to an alteration in pulsatile LH secretion (Knobil and Hotchkiss, 1988), although characteristics of the ovarian LH receptor have been reported to change with the phase of the cycle in *M. fascicularis* (Yoshida *et al.*, 1987).

It has also been proposed that intraovarian PGs are involved in luteal regression (Auletta *et al.*, 1984a). Although the CL of the primate is thought to be insensitive to PGs, studies in marmosets have shown that a single intramuscular injection of 0.5 mg cloprostenol (a $PGF_{2\alpha}$ analog) between Days 10 and 17 of the ovarian cycle results in sudden demise (Hearn, 1986; Summers *et al.*, 1985). Peripheral blood progesterone fell within 24 hr of treatment. However, although an effective luteolytic agent in marmosets, it is ineffective when administered to baboons (Eley *et al.*, 1987).

It has been speculated that estrogens can induce luteolysis in rhesus monkeys by acting directly within the ovary containing the CL; the proposed mechanism of estradiol-induced luteolysis may also be mediated through intraovarian PGs or through an inhibitory effect on progesterone synthesis. It has also been suggested that estrogens may be the biochemical signal responsible for the synthesis and release of ovarian $PGF_{2\alpha}$ and oxytocin, and that oxytocin of luteal origin may play a role in spontaneous luteolysis in the rhesus monkey (Auletta *et al.*, 1984b; Hodgen and Itskovitz, 1988).

Maintenance of the CL is uniquely dependent on continued gonadotropin support. The interruption of LH secretion at any point during the luteal phase results in a rapid decline in progesterone and sloughing of the endometrium within 2 to 5 days. The hormonal patterns and menstrual cycle lengths of Old World monkeys and great apes are strikingly similar, with both the follicular and luteal phases of approximately equal length. It should be noted that alternations in cycle length are usually the result of changes in the follicular phase, as the length of the luteal phase will usually remain constant.

c. ENDOMETRIAL CHANGES. The production of steroid hormones by the ovary cause significant changes in the uterine endometrium. The basal layer (*lamina basalis*) is never lost whereas the functional layer (*lamina functionalis*) is shed and regenerated each month. The first day of menses is considered the first day of the cycle. Generally, the menstrual phase lasts from 3 to 5 days and is followed by the proliferative phase which lasts about 9 days. The endometrium thickens two- to threefold, the spiral arteries elongate, and the glands increase in number. During the secretory phase (13–14 days in length), the production of progesterone by the CL stimulates the endometrial glands to secrete a glycogen-rich material and to become tortuous and develop a saccular appearance. The spiral arteries continue to grow and become more coiled. Studies have shown that only a specific relationship of progesterone:estradiol will support development of the normal secretory endometrium. In rhesus and long-tailed macaques, the luteal–follicular transition is a period characterized by diminishing progesterone levels while estradiol remains virtually unchanged (Goodman *et al.*, 1977).

Under normal circumstances, if fertilization occurs, implantation will occur on Day 21 or 23 of the cycle. If fertilization

does not take place, the secretory endometrium enters an ischemic phase as the spiral arteries constrict from the reduced production of progesterone by the regressing CL. Hormonal withdrawal also results in a cessation of glandular secretion, loss of interstitial fluid, and a shrinking of the endometrium. Readers are referred to reviews regarding the histologic changes in the endometrium which occur during the menstrual cycle (Brenner and Masler, 1988; Padykula, 1988; Padykula *et al.*, 1989).

d. SEASONALITY. The effects of changing season have been recognized in some species of nonhuman primates, particularly the rhesus (Drickamer, 1974; Hartman, 1932; Michael and Zumpe, 1971; Roberts, 1978; Vandenberg and Vessey, 1968), although the mechanism for this effect is unknown. Environmental cues have been linked to a decreased mating frequency, an increased variability in cyclicity, and a reduction in fertility, although most of these factors are still affected in controlled environments (Dailey and Neill, 1981; Riesen *et al.*, 1971). Influential factors may include temperature, humidity, and photoperiod in addition to food availability. In rhesus macaques, the majority of fertile cycles occur between October and December, a period of decreasing or short daylight (Roberts, 1978; Vandenberg and Vessey, 1968). However, experimental alterations in the light–dark cycles for indoor-housed macaques indicate that light alone does not regulate seasonal breeding in this species (Wehrenberg and Dyrenfurth, 1983). Further studies have investigated the effect of season on ovarian folliculogenesis, namely morphological and hormonal changes associated with the development of the DF (Hutz *et al.*, 1985). Results indicate a reduction in the percentage of cycles in which a DF could be observed via laparoscopy during May through August, which suggests that folliculogenesis was impaired. In some animals, however, it was noted that follicular development was delayed, not prevented, and that ovulations did occur. It was concluded that variations in the development of follicles could be responsible for reduced fertility in both natural and artificial environments.

A second hypothesis which was proposed centers around alterations in GnRH pulse frequency and gonadotrope sensitivity to GnRH or steroid hormones. It has been suggested that an alteration in pulse frequency or sensitivity may specifically be affected during the summer months. The importance of the pituitary in this regard is, however, questionable since the administration of estradiol benzoate during the summer months will elicit FSH and LH surges (Dailey and Neill, 1981).

IV. PREGNANCY

The critical events which must occur postfertilization in order to ensure the survival of the conceptus include implantation in the uterus and the "rescue" of the CL by chorionic gonadotropin (CG). Both of these events involve a receptive uterine environment and the establishment of the trophoblast. Generally speaking, there is a narrow window during which implantation can be initiated, although existing data suggest that the requirements for synchrony between the embryo and uterus are less rigid for primate than for the murine species (Hearn, 1986).

Most of the macaques and larger species have singleton pregnancies, with twin pregnancies being a rarity (Gould and Martin, 1981; Tarantal and Hendrickx, 1988). This is unlike marmosets and tamarins, who usually have dizygotic twins, as a rule. Growth and development of the conceptus during both the embryonic and fetal stages have been well described and are discussed in Chapter 14.

A. Prosimians and New World Species

1. Implantation and Placentation

A common characteristic of all prosimians is the existence of a bicornuate uterus and an expanded blastocyst at the time of implantation. In the case of the mouse lemur, the blastocyst fills the uterine lumen. The inner cell mass (ICM) is orthomesometrially located and is not covered by the trophoblast. The mouse lemur is the only species among the Lorisidae where a portion of the trophoblast invades the stroma; however, the greater part of the uterine and trophoblastic relationship is epitheliochorial. In the lesser galago, the blastocyst is oriented similar to that of the mouse lemur at the initiation of implantation, although when the blastocyst is approximately 0.5 mm in diameter an abembryonic attachment plaque forms. This pattern of an exposed ICM and an antimesometrial absorption plaque is also present in the loris. In the tarsier, an intermediate implantation characteristic occurs, namely trophoblastic invasion, which is midway between the development of other prosimians and the higher primates. Implantation in the prosimian and New World species has been reviewed by Enders and Schlafke (1986).

In prosimians, the placenta is of the epitheliochorial type [i.e., the fetal tissue (chorion) does not invade or remove maternal tissue] and the chorionic villi extend over the entire surface of the chorion where it is in direct apposition to the uterine epithelium. The villi are much less elaborate in form in that the degree of branching is limited and may consist of irregular folds in both the chorion and uterine lining which is typical of a "diffuse" placenta. There are several exceptions to this general type of placentation such as noted in the demidoff galago. The invasive development observed in the mouse lemur also occurs in this species, which provides a mixed epithelio- and endotheliochorial placentation. The fine structure and comparative aspects of placental development in the prosimians have been reviewed (Benirschke and Miller, 1982; King, 1984).

Implantation and early placental development have only been studied in a few species of New World primates. Timed collections from marmosets suggest that implantation may not occur until Day 11 or 12 (Table VI). The ultrastructural morphology of initial implantation stages has been studied in this species at known intervals after ovulation (Smith *et al.*, 1987). A true

TABLE VI

PRINCIPAL STAGES OF IMPLANTATION AND APPROXIMATE TIMING (DAYS) AFTER OVULATION IN HUMANS AND PRIMATES[a]

Stage	Human	Baboon	Rhesus	Marmoset
Embryo enters uterus	3–4	4	4	4
Zona pellucida shed	5–6	7–8	7–8	9–10
Attachment	6–7?	8–10	8–10	11–12
Trophoblast differentiates (previllous stages)	7–12	10–25	10–30	12–40
Tertiary villi	16–17	23–25	23–25	40+
Decidual reaction	12+	Local edema (11–14)	Plaque (11–14)	Epithelial 20+ Stromal 30+
Implantation type	Interstitial	Superficial	Superficial	Superficial

[a]From Hearn (1986).

hemochorial placenta develops following a superficial implantation. Initially, attachment is restricted to the embryonic pole of the blastocyst. Beneath the ICM the syncytiotrophoblast intrudes between the endometrial epithelium, although there is little response demonstrated by the maternal tissues to this invasion. Several days later, trophoblastic invasion has displaced the endometrial epithelium from its basal lamina in the area underlying the blastocyst; in this way the marmoset resembles the implanting rhesus monkey embryo. By Day 19 of gestation, both cyto- and syncytiotrophoblast extend almost entirely around the uterus, a process which is completed by Day 31 postovulation. This lateral extension of the trophoblast provides a large surface area in close proximity to the maternal circulation which is believed to provide adequate nutrients for the early embryo. The syncytiotrophoblast invades the stromal tissue adjacent to the maternal blood vessels, but separation from the maternal circulation is maintained by basal laminae, stroma, and endothelial cells lining the maternal blood vessels. Cytotrophoblast column formation is first evident by Gestational Day (GD) 23; around the deeper blood vessels there is a mild decidual response as indicated by hypertrophy and hyperplasia of the stromal cells. Further development of the cytotrophoblast is more evident at Day 31 postovulation as the columns extend into the syncytiotrophoblast between adjacent maternal blood vessels. The mesodermal layer, first observed on GD 19, forms early placental blood vessels, indicating the earliest differentiation of primitive villi. As will be noted in the following discussion, chorionic villi formation in the marmoset occurs much more slowly than in the rhesus monkey or baboon.

The exact time of implantation in the squirrel monkey is not known, although expanding blastocysts have been recovered from the uterine lumen 5 days after mating, and implantation has been shown to occur shortly thereafter (Ariga and Dukelow, 1977). Reports indicate that the zona pellucida of the squirrel monkey might be lost while at the <107 cell stage, which could imply that entry into the uterus and development of the embryo occur much earlier when compared to other primate species. After the initial penetration of the maternal endometrium, continued trophoblastic penetration is slow. Chorionic mesoderm formation occurs prior to invasion of the maternal vessels. With further development, the syncytial trophoblast increases in mass and surrounds maternal capillaries, forming a series of labyrinthine spaces into which maternal blood will enter (Enders and Schlafke, 1986). In the case of the howler monkey, which develops a monodiscoid placenta, there is a large amount of syncytial trophoblast at the earlier stages. This syncytium partitions the blood-filled spaces of the trophoblastic plate into a series of lacunae. In this species, the first formation of the cytotrophoblast columns plays a less important role in determining the placental arrangement than in some of the higher species. New World monkeys develop villi which become disc-shaped around the body stalk (future umbilical cord) in addition to the opposite pole, in some species. Thus, it is known as a "discoid" placenta and contains both the embryonic and maternal elements which serve as a medium of exchange.

2. Endocrine Changes

In the ruffed lemur, total urinary estrogen secretion is low during the second half of gestation and increases steadily, reaching values roughly 1000 times greater than those of estrous females. Estrone is the major estrogen formed in this species, with lesser amounts of estradiol. New World monkeys, including the marmoset, are unique in that the CL of early pregnancy is not a source of estradiol. There is indirect evidence that demonstrates the importance of the fetal adrenal in this regard.

CG is detectable in the urine of owl monkeys from 16 weeks prepartum until birth, whereas CG in the marmoset can be measured in the peripheral plasma from Days 10 to 15 of pregnancy and has been shown to increase rapidly until its peak at 6 to 8 weeks. For the squirrel monkey, CG can be detected between 20 and 105 days postconception. Little is known regarding the timing of the shift in steroidogenesis from the ovary to the placenta in prosimians (luteal–placental shift; see Section IV, B), although this event is believed to occur relatively late in gestation in the marmoset.

3. Twinning

The occurrence of twins varies greatly among the prosimians. The slender and slow loris show no evidence of twinning whereas the greater bushbaby has a twinning rate of 12–15%. In the lesser galago, a 59% twinning rate has been reported; among the lemurs, twins and triplets are common. The ruffed lemur falls in the category of "litter-bearing" with 75% of all births resulting in twins and triplets and an average litter size of 2.6 young per female.

TABLE VII

LENGTH OF GESTATION FOR NONHUMAN PRIMATES

Species	Gestational length (days)
Prosimians	
G. crassicaudatus	132
G. moholi	139–146
L. catta	120–135
L. macaco	120–135
L. mongoz	126–135
L. tardigradus	166–169
M. murinus	59–62
N. coucang	192
Tarsius	180
V. variegata	99–106
New World species	
A. trivirgatus	120–140
Alouatta	191
Callithrix	141–146
Cebus	180
S. sciureus	155
Saquinus	140
Old World species	
M. mulatta	165–175
M. fascicularis	155–165
M. cyclopis	155–165
M. radiata	165–170
Papio	173–193
Great apes	
P. troglodytes	210–270
P. pygmaeus	210–275
G. gorilla	251–265

4. Length of Gestation

The lengths of gestation for a number of prosimians and New World species are listed in Table VII.

B. Old World Species and the Great Apes

1. Implantation and Placentation

The process of invasion of the uterus by an unattached blastocyst results in the development of an intimate relationship which involves a number of steps, namely: (1) apposition, (2) adhesion, (3) penetration of the uterine luminal epithelium, (4) basal lamina penetration, and (5) tapping of maternal blood vessels; however, not all of these processes have been studied in detail in the nonhuman primate (Enders and Schlafke, 1986). A number of reviews describe the events associated with implantation and early embryonic development in a variety of the Old World species (Enders and Schlafke, 1986; Hendrickx, 1971; Weitlauf, 1988), and, therefore, only an overview of the morphological characteristics will be described herein.

The rhesus blastocyst appears as a balloon on the surface of the endometrium on GD 9.5, and a column of syncytial trophoblast penetrates to the endometrial basal lamina (Table VI). Uterine epithelial cells are partially isolated and surrounded by flanges of the syncytial trophoblast. Soon thereafter (GD 10) an epithelial plaque reaction occurs in the uterine epithelium which may persist for up to 3 weeks. The cytotrophoblast increases in volume at the implantation site and intermixes with syncytium which has developed microvillous-lined clefts. By GD 10.5, lacunae (blood spaces) containing maternal blood develop and the basal lamina of the maternal blood vessels are penetrated by syncytial trophoblast. As a result of vascular penetration, maternal blood comes in contact with trophoblastic tissue, particularly lacunae which are lined by syncytial trophoblasts with numerous microvilli. The lacunae expand, and in the process, the implantation site becomes superficial as opposed to the interstitial implantation noted in the human (Enders and Schlafke, 1986). Trophoblastic villi form which are then filled with mesoderm-derived blood vessels and cells which establish a circulation within the conceptus by the beginning of the fourth week. As noted earlier, this is in sharp contrast to that observed in the marmoset where chorionic villi are not recognized until later in development.

Implantation in the baboon has characteristics which resemble the rhesus monkey, including the formation of a trophoblastic plate, the presence of an epithelial plaque reaction, and pronounced endothelial hypertrophy and hyperplasia, in addition to peripheral subepithelial edema. Early formation of trophoblastic lacunae is noteworthy and may attribute to the partial but transient eversion in early placental formation. Some cellular swelling may be observed, as noted in the rhesus, but neither develop the decidual reaction observed in the human, where extensive changes in the endometrial connective tissue can be seen (Table VI).

Old World monkeys and apes have a mono- or bidiscoid placenta; the rhesus and long-tailed monkey usually have a bidiscoid placenta with single discs occurring roughly 20% of the time. The usual locations for the discs are on the ventral and dorsal walls of the uterus but several variations in location may be observed. In contrast to the rhesus monkey, the baboon will usually display a monodiscoid placenta, although in the early stages of pregnancy evidence of a second implantation site (i.e., secondary disc) may be present. In all species, bidiscoid placentas are interconnected by interplacental blood vessels which are located within the membranous chorion (King, 1986).

Placental weight in the rhesus monkey increases linearly with gestation and the ratio of placental:fetal weight changes from 10:1 in early pregnancy to 1:4 near term. Although the placenta continues to increase in weight throughout pregnancy, the maximum rate of both placental and fetal growth is between 125 and 150 days of gestation, with the maximum weight attained by GD 145. An increase in DNA content will also occur in a linear fashion during gestation.

Very little is known about early placental formation in the great apes. Only a single implantation site has been reported in the chimpanzee which was noted during the late previllous

stage of development (Enders and Schlafke, 1986). The placenta in Pongidae is considered to be remarkably similar to humans; it is discoidal, villous, and hemochorial in structure. Decidualization is also comparable to that observed in humans (Hartman, 1939; Soma, 1988).

The development of a functional placenta is required for the adequate exchange of gases, organic and inorganic materials, water, and nutrients, all necessary for the survival of the conceptus. Although described as a "selective" barrier, a variety of substances such as pharmacologic compounds, in addition to bacteria and viral particles, can readily cross the placenta and enter the embryonic/fetal compartment. Transmission may be accomplished by simple facilitated diffusion, active transport, solvent drag, phagocytosis, or pinocytosis and can occur at any location where maternal and fetal tissues are contiguous.

Of interest is the characteristic "placental sign" (implantation bleeding) normally observed in the rhesus and long-tailed macaque (Hartman, 1932; Jewett and Dukelow, 1972a). The occurrence of vaginal hemorrhage at approximately the same time that normal menstruation would appear negates its usefulness as a reliable indication of pregnancy. Hartman (1932) noted that the duration of the placental sign ranged from 13 to 32 days in the rhesus, with a mean occurrence of 23.3 days; half of the observations were noted between 21 and 26 days. Virtually 100% of the animals monitored displayed vaginal hemorrhage with early pregnancy, many as early as GD 14. It was also observed that very light to heavy bleeding occurred either uninterrupted for several days or reappeared with several occurrences during GD 14–50. Implantation bleeding has also been observed in *M. fascicularis* with initiation on Days 18–21, and a 17-day duration (Hartman, 1932). Comparative studies with macaques including *M. mulatta, M. fascicularis,* and *M. arctoides* indicate that all display a placental sign, although only 50% of stump-tailed macaques showed vaginal hemorrhage in contrast to 93% (long-tailed) and 76% (rhesus) for the other species. The onset of vaginal hemorrhage occurred on GD 19.2 ± 0.8 (long-tailed), 20.2 ± 0.2 (rhesus), and 16.8 ± 1.8 (stump-tailed). Similar findings have been reported by Jewett and Dukelow (1972a), although the duration for *M. fascicularis* was considerably shorter (4–9 days). Implantation bleeding does not appear to be a common occurrence in the larger species.

2. Endocrine Changes

The hormonal patterns during pregnancy for the rhesus monkey are shown in Fig. 3. In order for pregnancy to be maintained during the early stages of development, the CL must remain functional in its steroidogenic capacity. In nongravid animals, the CL normally regresses approximately 2 weeks after it develops. Its life span is extended (and it is 'rescued') once implantation occurs and the syncytiotrophoblast begins to secrete CG (Knobil, 1973). The importance of the CL during this period of gestation has been shown in studies where removal during this stage of development has resulted in sudden pregnancy termination (Goodman and Hodgen, 1979). Evidence which further supports CG as the signal for rescue of the CL comes from experiments by Neill and Knobil (1972) where treatment of nonpregnant rhesus females with human CG (hCG) during the luteal phase resulted in a prolongation of menstrual onset.

The initial detection of CG peripherally has been noted ~12 days postovulation in the rhesus (Atkinson *et al.*, 1975) and baboon (Shaikh *et al.*, 1978), and 11 days postovulation in the chimpanzee (Reyes *et al.*, 1975). In contrast to the chimpanzee (Clegg and Weaver, 1972; Nixon *et al.*, 1972), orangutan (Czekala *et al.*, 1981, 1983), and gorilla (Tullner and Gray, 1968), CG levels in the rhesus remain elevated for a very short period of time (about 5 to 10 days; GD 17–26); CG cannot be detected in blood or urine after GD 40 (Hobson *et al.*, 1975; Hodgen *et al.*, 1972; Hodgen and Tullner, 1975). This is similar to other macaques such as the long-tailed (Hein *et al.*, 1989) and pig-tailed (Chandrashekar *et al.*, 1980), where transient levels of CG were observed in early pregnancy, with a peak in production on ~GD 22–24, and titers nondetectable greater than GD 40. Studies by Hobson and Wide (1981) indicate that, contrary to prior studies, CG can be detected in term placentas from a variety of New World (owl and spider monkey, marmoset, lemur) and Old World species (rhesus, capuchin), in addition to the baboon, gibbon, chimpanzee, orangutan, and gorilla. The role of CG during the third trimester of pregnancy remains to be elucidated.

The ovary remains critical until steroidogenesis, namely progesterone production, is taken over by the placenta. The role of progesterone in pregnancy maintenance and its relationship to the estrogens have been well established. Estrogens do, however, provide support for this function by generating and maintaining progesterone receptors both within the uterus in addition to other target tissues. Interestingly, the production of estrogens does not appear to be as critical during this period, as little change in concentration is noted. Secretion of progesterone from both the CL and fetoplacental unit occurs simultaneously until approximately the third week of pregnancy, when CG levels decline and progesterone and estradiol increase. This change in production of hormones from the ovary to the placenta is termed the luteal–placental shift (LPS) and emphasizes that the placenta is responsible for hormonal support for the remainder of pregnancy, in conjunction with the interaction of the embryo/fetus (Hearn, 1986). Neither hypophysectomy (Smith, 1954) nor ovariectomy (Hartman, 1939; Tullner and Hertz, 1966) will induce abortion once the placenta has become established in this role. The substantial placental production of progesterone occurs during ~GD 14–21 in the rhesus (Bosu and Johansson, 1975) and GD 20–25 in the baboon (Castracane and Goldzieher, 1986), indicating the timing of the LPS.

Evaluation of the secretory dynamics of both the ovary and placenta during the LPS have led to the following conclusions: (1) although the CL loses its capacity to secrete progesterone during the early stages of pregnancy, it develops a greater ability to form estrogens which is the result of increased androgen secretion and enzyme activity; (2) estrone is the primary estrogen secreted by the CL in early pregnancy whereas estradiol becomes primary during the later stages; and (3) the placenta

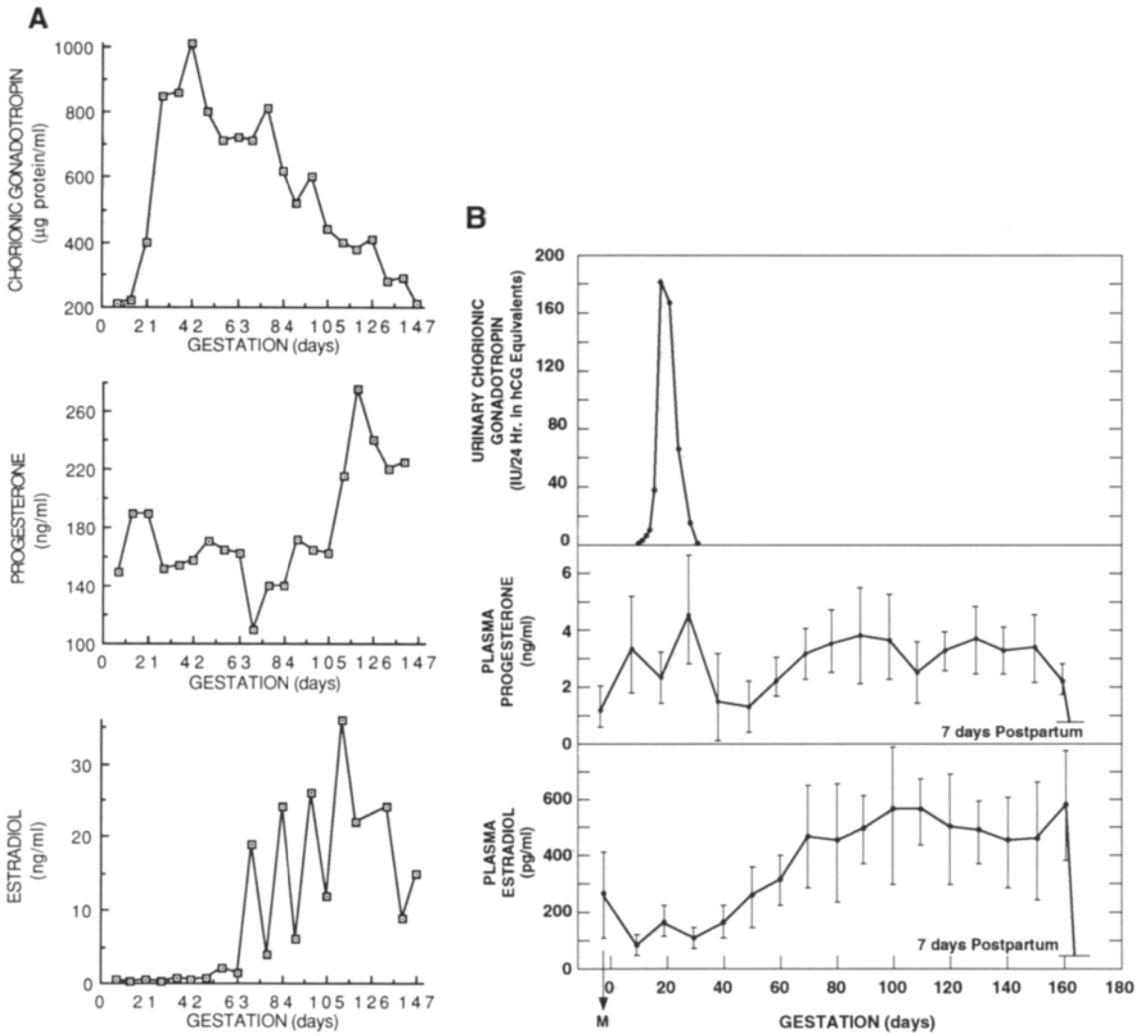

Fig. 3. Composite of concentrations of urinary macaque chorionic gonadotropin, and peripheral plasma progesterone and estradiol from pregnant rhesus monkeys from day of mating (M) until 7 days postpartum. From Hodgen *et al.* (1972). Copyright © 1972, The Endocrine Society.

synthesizes progesterone as early as GD 23, but is unable (via aromatase) to convert androgens to estrogens until the sixth week (Ellinwood *et al.*, 1989). The mechanism for the eventuality of CL regression during early pregnancy appears to be related to a loss of CG receptors with subsequent impairment of the pathway for progesterone biosynthesis, although studies have shown that although a significant decline in the number of available CG receptors occurs during simulated pregnancy, no net change in total receptor number is observed (Ottobre and Stouffer, 1986). This lack of down regulation has also been supported by studies by Monfort *et al.* (1989). The CL is capable of being rejuvenated at term, which indicates that regression–function is a reversible process (Koering *et al.*, 1973a,b).

In the rhesus, progesterone fluctuates between 3 and 4 ng/ml during the second and third trimesters (roughly 60–165 days), which is similar to the concentrations detected during the luteal phase of the menstrual cycle (Ducsay *et al.*, 1985; Hodgen *et al.*, 1972; Thau *et al.*, 1977). Serum progesterone plateaus in baboons at ~12 ng/ml between GD 60 and 184 (term), which is roughly twofold greater than the concentrations produced during the luteal phase (Albrecht and Townsley, 1976; Henson *et al.*, 1987). In the chimpanzee, progesterone rises progressively to 49–120 ng/ml at term (~GD 240) (Reyes *et al.*, 1975). Since the metabolic clearance rate of progesterone does not differ in pregnancy when compared to the nonpregnant state, the increase in serum concentrations observed throughout gestation reflects an increase in production rather than metabolism (Albrecht and Pepe, 1990).

During human pregnancy, large quantities of estriol are produced by the placenta, which is due to the presence of the 16α-hydroxylase enzyme within the fetus (Milewich *et al.*, 1986). Although the great apes share this feature (Czekala *et al.*, 1983; Reyes *et al.*, 1975), this is in direct contrast to the macaques

and baboon, while little or no estriol is formed and estradiol is the major circulating estrogen (Laumas, 1965; Solomon and Leung, 1972). Although this is a distinct difference between these species, a commonality observed is the elevation of estrogens which occurs throughout pregnancy in all species evaluated.

Increasing concentrations of serum estrone and estradiol occur in the rhesus with advancing gestational age; peak levels of ~0.4 and 0.7 ng/ml, respectively, have been noted at term (Bosu *et al.*, 1973). In the baboon, serum estrone and estradiol rise with increasing GDs, with total estradiol levels reaching a peak of 4–7 ng/ml (Albrecht *et al.*, 1989), which is 10-fold greater than the rhesus. In chimpanzees, serum estrone, estradiol, and estriol increase progressively during gestation, with term values peaking at 2–3, 5–8, and 4–10 ng/ml, respectively (Reyes *et al.*, 1975). For the orangutan and gorilla, total urinary estrogens increase linearly with advancing age and achieve values of 2.2–3.9 ng/ml and ~30 μg/mg urinary creatinine, respectively, at term (Hopper *et al.*, 1968; Czekala *et al.*, 1983). The fetal adrenal appears to play a significant role in the biosynthesis of placental estrogens; readers are referred to reviews describing these biosynthetic pathways and steroid and polypeptide hormone regulation (Albrecht and Pepe, 1988, 1990; Solomon, 1988; Talamantes and Ogren, 1988).

Studies in the long-tailed monkey indicate that serum levels of CG and estradiol are similar to the rhesus (Fujiwara *et al.*, 1980; Hein *et al.*, 1989). In contrast to prior studies where progesterone and CG peaked coincidentally, progesterone peaked prior to CG. Peripheral concentrations of progesterone on GD 10 in the pig-tailed macaque have been measured and were found to be 13.0 ± 2.2 ng/ml, which declined progressively to 8.0 ± 1.3 ng/ml by GD 21 (Chandrashekar *et al.*, 1980). Levels increased on GD 27 with a peak on GD 37 (18.5 ± 2.5 ng/ml), which declined to a nadir during midgestation.

In the African green monkey, plasma progesterone concentrations have been reported to peak at >20 ng/ml during the first month of pregnancy, which declined to a plateau of 5–7 ng/ml throughout the remainder of gestation (Hess *et al.*, 1979). As noted with other species, total plasma estrogens rise throughout pregnancy and attain peak concentrations of ~7 ng/ml at term with estradiol levels at a low of 80 pg/ml. Germain *et al.* (1988) studied placental and myometrial levels of estrone, estradiol, and progesterone in the long-tailed macaque at various stages of gestation. It was noted that steroid levels in the myometrium (ng/g wet weight) were similar to the plasma values, whereas placental levels of estradiol and progesterone were 4 times and estrone 10 times greater than myometrial levels. Myometrial profiles indicated a decrease of estrone during GD 80–130, whereas estradiol and progesterone were on the rise; placental profiles increased from GD 30 to GD 160.

3. Twinning

The incidence of live-born twins in Old World monkeys is extremely rare. A compilation of reports in the literature indicate a twinning rate of 0.2% (10/4991) for the rhesus (Tarantal and Hendrickx, 1988) and 0.07% (2/2867) for the long-tailed macaque (Korte *et al.*, 1988; Resuello, 1987). Sonographic studies indicate that the twinning rate may be greater than reported since the appearance of twin GS, one with a viable embryo and one anembryonic similar to the "vanishing twin" observed in humans, has been noted (Tarantal and Hendrickx, 1988) (see Chapter 14).

4. Length of Gestation

The duration of gestation is similar in most Old World species (Table VII). *M. mulatta* (Hartman, 1932) and *M. radiata* (Stabenfeldt and Hendrickx, 1972) usually deliver during GD 165–170, although normal unassisted deliveries have occurred through GD 175. Interestingly, Riopelle and Hale (1975) have noted that rhesus monkeys maintained in a semioutdoor environment may show alterations in the length of gestation which appeared to be dependent on the protein concentration of the diet, weight at conception, and season of delivery. These data imply that for those species that display seasonality, a number of environmental factors may control the timing of parturition. Generally, most macaques deliver during the late evening or early morning hours, which is preceded by a labor duration of 2 to 3 hr (Goodlin and Sackett, 1983; see Section V).

For *M. fascicularis* (Boot *et al.*, 1985; Gardin *et al.*, 1989; Jewett and Dukelow, 1972a), deliveries have been reported to occur during GD 155–165, which is similar to *M. cyclopis* (Peng *et al.*, 1973) and the talapoin monkey (Rowell, 1977b). The baboon has been reported to deliver during ~GD 173–193 (Gilbert and Gillman, 1951). The duration for the great apes has been noted for the chimpanzee, orangutan (Schwartz, 1988), and the gorilla (Faiman *et al.*, 1981) and occurs during ~GD 210–270, 210–275, and 251–265, respectively (see review by Ardito, 1976). Much of the variation noted in these reports is due to a lack of a fixed conception date.

V. PARTURITION

Parturition, the act of giving birth, is the result of a variety of endocrinologic and physiologic factors involving both the maternal and fetal compartments, although the initiator(s) of these events is poorly understood. This physiologic cascade has been studied extensively; references have been provided as supplemental reading for this active and important area of research (McNellis *et al.*, 1988).

A. Prosimians and New World Species

Here, as with other aspects of gestation, there are no definitive findings with either prosimians or New World monkeys.

B. Old World Species and the Great Apes

Hormonal Mechanisms

The essential aspect of delivery is the development of rhythmical and coordinated contractions of the uterine musculature in conjunction with dilatation and effacement of the cervix. The uterus is rarely in a quiescent state and displays contractile activity (*contractures*) throughout gestation (Nathanielsz *et al.*, 1988). Several factors have been considered to play a role in the transition from contractures (which begin ~GD 80 in the rhesus) to the functional contractions of labor and delivery. These include the quantity of estrogen receptors, enhancement of the production of stimulatory versus inhibitory factors, changes in resting membrane potential, increased coupling of excitation and contraction, improved synchronism between myometrial cells in the transmission of these signals, and the coordination of myocyte contraction. The relative quiescence of the uterus during most of gestation is attributed primarily to progesterone which may uncouple uterine activity by binding calcium more tightly to membranes and may suppress the synthesis of PGs, a significant component in the cascade, either within the myometrium or decidua and perhaps the membranes. Estrogens are most likely involved in promoting uterine contractile activity by their action on myometrial contractile protein synthesis, receptor and gap junction regulation, and PG formation in the myometrium, decidua, and fetal membranes (Challis and Olson, 1988). The uterine actions of estradiol are, therefore, somewhat antagonistic to the quiescent effects of progesterone. Alterations of the ratio of estradiol:progesterone may be involved in the physiologic cascade of parturition, although in primates, no apparent change in the estradiol:progesterone ratio with the resultant trigger of PG release has been noted.

As discussed in the section entitled "Pregnancy" (see Section IV,B,2), ovariectomy can be performed in the macaque during pregnancy without risking abortion, provided it is performed after the LPS occurs. This implies, therefore, that the ovary plays an insignificant, if any, role in steroidogenesis at this stage. Although plasma progesterone remains at roughly luteal phase levels during the second half of gestation in the rhesus (~2–4 ng/ml), an increase in the ovarian production of progesterone to 6–8 ng/ml occurs near term (Walsh, 1985). Interestingly, it has been shown that the functional capacity of the CL is retained throughout gestation and lactation (Chandrashekar *et al.*, 1980; Hodgen *et al.*, 1977b; Weiss *et al.*, 1973), although the role of extended luteal function remains to be elucidated. Implications of a "rejuvenation" of the CL function (Koering *et al.*, 1973b; Stouffer *et al.*, 1977) are supported by findings of higher concentrations in the ovarian vein draining the ovary containing the CL (Walsh, 1985). The CL of the postpartum macaque remains steroidogenically active, although the nocturnal rhythmicity observed prior to parturition has not been detected postdelivery. In most other nonhuman primate species evaluated, progesterone concentrations appear somewhat greater than the rhesus, although none of the species studied indicate that progesterone is involved in the initiation of labor. The chimpanzee shows a moderate increase at term with concentrations ~100 ng/ml. *M. fascicularis* maintains low and stable levels of progesterone up through the last week of pregnancy, whereas *M. radiata* shows a 10-fold increase prior to delivery (Stabenfeldt and Hendrickx, 1972).

The concentrations of circulating estrogens are somewhat lower in the rhesus when compared to other nonhuman primates. However, this does not imply that the biological actions are different, especially when considering that peripheral steroid levels are not necessarily representative of local production (Walsh, 1985; Germain *et al.*, 1988). During the second trimester, estradiol rarely exceeds estrone and usually will not achieve levels greater than 1 ng/ml; maternal estrone and estradiol both rise late in gestation. Plasma concentrations of estradiol are unchanged by ovariectomy during late pregnancy but fall after fetal death, fetectomy, or administration of dexamethasone to the dam or fetus, implying that it originates from the fetoplacental unit and that the fetus plays a major role in its production. In the chimpanzee, maternal estradiol levels increase gradually during pregnancy to 5–8 ng/ml at term, although no terminal increase is observed (Lanman, 1977). The issue of maternal estrogens serving as a trigger for parturition in the monkey is not well-defined, although there is some evidence suggesting a potential role. Walsh *et al.* (1984) have studied the hormonal changes in the rhesus maternal, fetal, and amniotic fluid compartments prior to parturition and noted that delivery was preceded by rising concentrations of dehydroepiandrosterone sulfate (DHEAS) in the fetal but not maternal compartment. Parallel increases in fetal plasma estrone, maternal plasma estrone and estradiol, and amniotic fluid estrone all preceded a rise in amniotic fluid $PGF_{2\alpha}$ metabolite. Although progesterone levels were maintained prior to delivery, studies with RU 486, a potent synthetic progesterone receptor antagonist, have shown an increase in estrogen receptors in the decidua and myometrium, but not in amnion or chorion, after administration to third trimester rhesus monkeys (Haluska *et al.*, 1990). This implies that the presence of progesterone in sufficient quantities is required in order to limit the number of estrogen receptors formed, thereby maintaining a quiescent uterine environment conducive to maintaining pregnancy. RU 486 is capable of inducing and increasing uterine contractility during late pregnancy, although it has not been shown to be associated with an increase in amniotic fluid PGs until 48 hr post-treatment (Haluska *et al.*, 1987). In addition, little cervical dilatation and effacement were noted.

In all species studied to date, PGs have been found to be the regulators of myometrial contractility and to serve as an essential component in the onset and progression of labor. In the primate, the myometrium appears to be sensitive to PGs at all stages of gestation. Measurements of amniotic fluid concentrations of leukotrienes and $PGF_{2\alpha}$ in conjunction with uterine activity prior to parturition indicate that all increase with labor onset (Walsh, 1989).

Relaxin and oxytocin are hormones of importance in primate pregnancy, although their roles have yet to be clearly defined.

Relaxin is produced by the CL, myometrium, decidua, and placenta, but not amnion of pregnant baboons (Castracane *et al.*, 1985). Highest levels are reached during the first trimester, although concentrations are still detectable in the circulation throughout gestation. Administration of exogenous relaxin has been shown to stimulate separation of the pubic symphysis and promote myometrial relaxation in addition to enhancing cervical ripening. Relaxin may also contribute to uterine quiescence by inhibiting oxytocin release. Controversy still exists over the precise role of oxytocin in relation to the initiation of labor. Significant concentrations have been found in both the maternal and fetal compartments; it is also well-established that oxytocin stimulates the production of PGs, specifically PGE_2 and $PGF_{2\alpha}$. In *M. fascicularis*, measurements of plasma levels of oxytocin during pregnancy and lactation indicate low levels throughout the third trimester with a peak on the day of parturition (22.4 $\pm$ 6.7 pg/ml) (Morris *et al.*, 1980) with concentrations returning to baseline levels during lactation. Oxytocin may also play a significant role in the switch from nocturnal contractures to contractions leading to labor and delivery (Honnebier *et al.*, 1989).

Issues related to management and potential problems that can arise during parturition are discussed in Chapter 14.

VI. LACTATION

Although the primary function of lactation is to supply nutritive substances to the young, a secondary role of particular importance in nonhuman primates is postpartum (lactational) infertility. In Old World monkeys and the great apes, the suppression of gonadotropin release appears to be responsible for this alteration in cyclicity (McNeilly, 1988), although inhibition at all three points of the hypothalamic–pituitary–ovarian axis has been postulated. This phenomenon accounts for the efficient spacing of births which is important from both a maternal and infant perspective.

Nonhuman primate infants begin to nurse shortly after birth, usually within minutes or hours postpartum. The stimulation of milk secretion is a well-known action of prolactin in most mammalian species with the infant's suckling as the primary stimulus, although other mechanisms may be involved (Blank, 1986). Prolactin has been shown to induce the production of major milk proteins in the mammary glands of both mature and immature monkeys and apes; in marmosets, an elevation of prolactin will even occur in males when assisting with the care of newborn infants.

A. Prosimians and New World Species

1. Length of Lactation

Slender loris females have four mammary glands (one pair in the pectoral region and one pair abdominally). It is possible to manually express milk from these glands from 153 to 172 days postpartum; milk can be found in the anterior aspect of the mammary glands up to 219 days with cessation of production by 231 days. In the slow loris, lactation lasts approximately the length of gestation, which is about a month longer than the slender loris and the galago. The lesser galago will nurse 70–90% during the first 2 or 3 days after birth and, by the second week, nursing will decrease to 30–50% with cessation at approximately 80 days postpartum. By the time the infants are 2 weeks of age, their size will triple. During the act of nursing, the dam will often change position which requires that the infants relocate the nipple without maternal assistance (Doyle *et al.*, 1969).

Length of lactation in the lesser galago is significantly shorter than that of *G. garnettii* or the greater galago (100, 139, and 136 days, respectively) (see Table VIII). Litter size does not appear to have an effect on the length of milk production (Izard, 1987; Izard and Simons, 1987). If infant death occurs, lactation will continue for an additional 11–15 days. The young marmoset will suckle for approximately 100 days, ingest solid food beginning approximately 30 days of age, and at $\geqslant$40 days can exist entirely on solid food and milk substitutes. Owl monkey infants will leave the dam at 22–46 days of age and begin to eat solid food between 35 and 60 days. The young represent 14–16% of adult body weight at birth and grow rapidly, which may represent a mechanism for species survival. By 5 weeks of age, the infant can weigh as much as 200 g, which is roughly 20% of the maternal body weight. By 4.5 months, most will be independent of the parents for the majority of the time (Dixson, 1983). In contrast to most of the Old World species, owl monkey infants will rarely remain on the nipple except for suckling. For marmosets, tamarins, and the owl monkey, care of the infant is actively shared by other family members.

2. Milk Composition

Gross compositions of fat, protein, lactose, and ash in the galago and squirrel monkey are listed in Table IX.

3. Milk Production

It is not fully understood how nutrition relates to milk production in nonhuman primates, although the literature indicates that lactation in the smaller species appears to require a higher energy/nutritional demand. These animals meet these greater requirements by increasing the rate of food intake and switching to higher-quality foods or by feeding for longer intervals (Lee, 1987). This would imply that milk production would be severely affected by malnutrition or by a decrease in quantity or quality of the daily diet. Studies which have centered around dietary restriction and its effects on lactation have shown that when nutrient intake is restricted to 80% of the normal *ad libitum* intake for lactating females, milk output is not significantly

TABLE VIII

LENGTH OF PRIMATE LACTATION[a]

Species	Age infant first eats solid food (months)	Age of weaning (months)
Prosimians		
A. calabarensis	1	2–3
A. laniger		5
C. major	1	1.5
G. crassicaudatus	0.5–1	3–5
G. demidovii	0.5–1.5	1–1.5
G. senegalenis	0.5–1	1.5–3.5
Hapalemur spp.		1.5
Indri spp.		4
Lemur spp.	1	5–6
L. mustelinus	1.5	2.5–4
L. tardigradus	1–1.5	6
M. murinus	0.5–1	1.5–5
N. coucang	0.5–2	3–6
P. potto		5
P. verreauxi		3–7
U. everetti	1	
New World species		
A. palliata		18–24
C. goeldii	1	2.5
Callithrix spp.	1	2–6
C. pygmaea	1.5–2	3
Lagothrix spp.		12
Leontideus spp.		2–4
S. geoffroyi	1–1.5	
S. sciureus	1–1.5	6
Old World species		
C. aethiops	2	6
C. (Miopithecus) talapoin	0.5–1	2–6
M. fascicularis	1	12–18
M. fuscata	1.5	6–8
M. mulatta	1.5–3	7–14
M. nemestrina	1	8–12
M. radiata	1	8–12
Papio spp.	3–6	6–15
P. entellus	3	10–15
Great apes		
P. troglodytes	4–6	18–42
P. pygmaeus	6	24–36
G. gorilla beringei	2.5–3	12–24

[a]Adapted from Buss (1971).

reduced, although restriction of the diet to 60% results in a reduction of milk output by roughly 20% (Roberts *et al.*, 1985).

4. Lactational Amenorrhea

Although many Old World species exhibit a period of postpartum (lactational) amenorrhea which impedes ovarian function and subsequent fertility, this is not evident in marmosets (Kholkute *et al.*, 1988; McNeilly *et al.*, 1981). In this species, neither suckling nor high levels of prolactin delay ovulation, even when highly elevated prolactin levels occur due to the presence of twins. Ovulation occurs between 10 and 18 days postpartum with increased sexual encounters at that time (Dixson and Lunn, 1987). Limited reports on the howler monkey indicate cyclical changes 3 to 4 months postpartum and while nursing. If the infant dies, females begin to solicit mating within 3 weeks. Under these circumstances, pregnancy can occur immediately (Glander, 1980). In the greater galago, postpartum estrus occurs as early as the second day after parturition but is usually 10 days later with a mean length of 23 days; 43% of postpartum estrus matings result in conception and 81% of these result in live births (Valerio *et al.*, 1972). The lesser galago has a postpartum estrus within 24 hr of parturition which usually results in mating (Doyle *et al.*, 1971), whereas the owl monkey does not display this characteristic.

For the slender loris, no immediate postpartum estrus occurs, although there is evidence of mating as early as 8 days after parturition. A postpartum estrus has been reported to occur within 4 to 9 days in the slow loris, although none have resulted in pregnancy (Izard *et al.*, 1988).

5. Interbirth Intervals

Hearn (1983) noted that longer interbirth intervals occur with those species whose infants display less maturity at birth in addition to longer periods of dependency (such as the great apes). In contrast, marmoset offspring are independent of the dam well before the next birth; therefore, lactation-induced infertility does not appear to be of importance. As an alternative, the marmoset maintains some degree of fertility regulation through the suppression of cyclicity in subordinate females (Hearn, 1984).

TABLE IX

GROSS COMPOSITION OF MILK FROM NONHUMAN PRIMATES[a]

Species	Composition (g/100 ml)			
	Fat	Protein	Lactose	Ash
Prosimians				
G. crassicaudatus	3.7	5.3	5.0	—
New World species				
S. sciureus	4.6	3.4	6.4	0.30
Old World species				
Cercocebus spp.	1.5	2.3	7.0	0.1
C. (Miopithecus) talapoin	2.9	2.1	7.2	0.28
C. sabaeus	4.0	3.1	10.2	0.6
M. mulatta	3.9	2.1	5.9	0.26
P. cynocephalus	5.0	1.6	7.3	0.26
Great apes				
P. troglodytes	3.0	1.0	7.1	0.21
P. pygmaeus	4.7	2.0	6.4	0.30
G. gorilla	2.2	1.1	—	—

[a]Adapted from Buss (1971) and other literature.

B. Old World Species and the Great Apes

1. Length of Lactation

The length of lactation varies with the species and the ability of the infant to gain dietary independence. At roughly 10 weeks of age, *M. fascicularis* infants will nurse for an average of ~210 min during the 8 daylight hours (Chance *et al.*, 1977). This is similar to the rhesus, baboon, and the bonnet (Hinde and Spencer-Booth, 1968; Hinde and White, 1974). Rhesus and baboon infants will place solid food in their mouths as early as 1 month of age, but will rarely ingest solid nutrients until 2 to 3 months. For animals reared with their mothers either in the laboratory or in the wild, the frequency of suckling has been reported to change gradually over the first 6 months to 1 year of age (Buss, 1971). Suckling may, however, continue for a more extended period and appears to be dependent on environmental factors such as birth of a sibling. It has been shown that infants of primiparous rhesus females suckle more frequently and make more nipple contacts than infants of multiparous females (Gomendio, 1989), although these findings are in direct contrast to an earlier study (Wilson *et al.*, 1988). Differences may be related to the methods used for analysis of the data.

Table VIII provides estimates of the length of lactation from observations in both the field and in the laboratory (Buss, 1971). Although there is little information available for the great apes, reports indicate that the duration of lactation, suckling, and amenorrhea may persist for several years (Nadler *et al.*, 1981).

2. Composition and Properties of Milk

Colostrum, the mammary gland secretion obtained within the first few hours of birth, is orange-tinged in squirrel monkeys; appears as a thin, opalescent liquid in baboons; and is a whitish, opalescent, stringy secretion in chimpanzees (Buss, 1971; Nissen and Yerkes, 1943). Milk has been obtained from both the chimpanzee and gorilla during the fourth to sixth month of pregnancy, and from the baboon prior to delivery following oxytocin administration. In contrast to the milk secreted during the latter stages of lactation, baboon colostrum is low in lactose and fat, contains more protein, is richer in immunoglobulin (Ig) A, and has less casein and ash concentrations (Buss, 1968; 1971). Concentrations of iron, copper, zinc, magnesium, sodium, potassium, and protein are also higher in the colostrum of rhesus females when compared to mature milk (Lönnerdal *et al.*, 1984; Table X), as are the Igs. Evaluation of the levels of IgG, IgM, and IgA in the plasma of infant Japanese macaques (*M. fuscata*) from birth to 6 months of age suggests that (1) the temporal increase in IgM may be related to the initial response by the neonates to antigen stimulation, and (2) the resistance against infectious diseases may be diminished at birth and subsequently decrease to lowest levels at ~60 days of age when body weights were 600–800 g (Takenaka, 1981).

The composition and nutritional content of *M. mulatta* milk have been well-summarized (Bourne, 1975; Lönnerdal *et al.*, 1984; Rassin *et al.*, 1978; van Wagenen *et al.*, 1941). Lönnerdal *et al.* (1984) studied milk compositions throughout lactation (Day 0 through >36 postpartum) with the following observations: iron concentration was high in early lactation (1.8 ± 0.8 μg/ml) and progressively decreased during the next 5 weeks (>36 days = 1.2 ± 0.2 μg/ml); copper levels dropped throughout lactation; calcium increased whereas protein decreased slightly; zinc decreased rapidly; and fat and lactose did not vary much with the stage of lactation. Milk obtained from postpartum long-tailed monkeys has been analyzed for gross composition and minerals over an average lactation period of 90 days (range of 44–119 days). Analyses indicated total solids of 12.2 ± 2.2 g/100 ml, crude protein 1.7 ± 0.3, lipids 5.2 ± 2.1, lactose 4.8 ± 2.3, ash 0.4 ± 0.1, sodium 95.8 ± 64.2 mg/100 ml, potassium 21.2 ± 12.8, magnesium 3.4 ± 1.3, calcium 38.7 ± 17.2, and phosphorus 15.2 ± 7.8 (Nishikawa *et al.*, 1976). Buss (1968) reported the gross composition of baboon milk for three species during the course of lactation (Days 0–279 postpartum) and the changes in milk composition during the first 14 days after removal of the infant; no differences were observed among *P. anubis*, *P. cynocephalus*, and *P. papio*. In early samples, the protein concentration was high and the lactose concentration was low; little change was noted in fat or ash during the sampling period. The lipid concentration, however, increased gradually as lactation progressed and was similar to values reported for the rhesus, chimpanzee, orangutan, and gorilla.

Primates are unique in that they have a higher proportion of calories present as lactose and a lower proportion as protein and fat (Buss, 1971). The fats are entirely triglycerides and contain palmitic, oleic, and linoleic acids, which are directly proportional to the maternal diet ingested. Other than the great apes, the mammary glands of nonhuman primates synthesize digestible fatty acids with 8–14 carbons in proportions which may vary with the species (Buss, 1971; Nishikawa *et al.*, 1976). Milk from the rhesus contains ~40 mg cholesterol/100 ml, which is twice that of *P. ursinus*, yet these infants show lower plasma cholesterol when nursing than when raised on cholesterol-free formulas (Buss, 1971). Milk protein concentration decreases only slightly with length of lactation; the casein:whey ratio is 1:4, which is similar to *M. fascicularis* (Nishikawa *et al.*, 1976). Although squirrel monkeys and chimpanzees appear to be more similar to macaques, the baboon, talapoin, and orangutan have more casein than whey proteins (Buss, 1971).

Concentrations of a number of free amino acids have been determined in various mammalian species (Rassin *et al.*, 1978, see Table XI). Taurine is the most abundant free amino acid in the rhesus and the second most abundant in the chimpanzee and long-tailed macaque. It was noted that the milk composition of each species evaluated had a characteristic amino acid pattern.

TABLE X

MILK COMPOSITION DURING LACTATION IN RHESUS MACAQUE, *Macaca mulatta*[a]

Nutrient	Days of lactation				
	0–5	6–15	16–35	36 →	Human mature milk[b]
Iron (μg/ml)	1.76 ± 0.84 (9)	1.21 ± 0.20 (15)	1.18 ± 0.22 (43)	1.15 ± 0.19 (37)	0.25 ± 0.03 (18)
Copper (μg/ml)	3.04 ± 1.09 (9)	1.21 ± 0.18 (15)[c]	0.60 ± 0.04 (43)[c]	0.46 ± 0.04 (37)[c]	0.27 ± 0.02 (18)
Zinc (μg/ml)	5.15 ± 1.64 (9)	2.41 ± 0.38 (15)[c]	1.84 ± 0.20 (43)[c]	1.86 ± 0.18 (37)	1.35 ± 0.13 (18)
Calcium (μg/ml)	350 ± 26 (9)	364 ± 27 (15)	420 ± 10 (43)[c]	392 ± 14 (37)[c]	270 ± 14 (18)
Magnesium (μg/ml)	49.6 ± 12.1 (9)	31.5 ± 3.6 (15)[c]	32.9 ± 3 (43)	30.7 ± 2.3 (37)	33.6 ± 1.1 (18)
Sodium (μg/ml)	171 ± 64 (9)	95.9 ± 17.7 (15)	89.4 ± 8.6 (43)	81.8 ± 6.5 (37)	184 ± 33 (18)
Potassium (μg/ml)	367 ± 33 (9)	242 ± 17 (15)[c]	260 ± 8 (43)	276 ± 10 (37)	470 ± 19 (18)
Protein (%)	2.49 ± 0.26 (5)	2.28 ± 0.09 (7)	2.34 ± 0.07 (13)	2.35 ± 0.09 (19)	1.32 ± 0.04 (18)
Fat (%)	5.29 ± 0.97 (4)	5.42 ± 0.51 (9)	4.57 ± 0.52 (17)	4.92 ± 0.50 (21)	4.58 ± 1.39 (18)
Lactose (%)	7.79 ± 0.13 (6)	8.06 ± 0.03 (8)	7.91 ± 0.06 (18)	7.92 ± 0.04 (25)	7.13 ± 0.18 (18)
kcal/100 ml	88.7	90.1	82.2	85.4	75.0

[a]Data are expressed as mean ± SEM. Numbers of samples are shown in parentheses. From Lönnerdal *et al.* (1984).
[b]Human milk values are based on data obtained in the author's laboratory. Mature milk was collected at 3 months postpartum.
[c]Significantly different from preceding time period ($p<0.05$).

3. Milk Production

The energy yield of primate milk, measured as the intake of protein and calories in a suckling bout, tends to be low in comparison with the milk of other mammals of similar size (Lee, 1987). Since energy is transferred at a lower rate in these species, it implies that a more lengthy lactational period would be required. The energetic requirements of lactation vary according to the changing rate of growth of the infant, with peak milk yield and metabolic demands during the phase of most rapid infant growth. For seasonal breeders under feral conditions, food supply may play a critical role in the timing of these events (Lee, 1987).

Accurate measurements of daily milk yield are difficult because the mammary glands of these species have a low storage capacity and the infants nurse frequently (Buss, 1971). Manual milking has been reported to yield as much as 9–10 ml in rhesus and greater than 20 ml from great apes. Baboons have been reported to secrete ~340 ml/day. Rhesus infants ingest similar amounts of formula, whereas prosimians may drink twice this amount (Buss, 1971).

4. Lactational (Postpartum) Amenorrhea

Lactation-induced infertility has been noted in a variety of nonhuman primate species, including the macaques, baboon, and great apes (McNeilly, 1988), and appears to be associated with a reduction in sexual activity in addition to ovulation inhibition. The majority of experimental studies focusing on gonadotropin regulation have been performed in the rhesus, therefore, most information available is based primarily on observations in this species. Reports indicate that FSH and LH remain suppressed postpartum and do not increase until 6 to 10 months (FSH) and 12 months (LH) later (Plant *et al.*, 1980). In contrast, nonsuckling females show a normal gonadotropin response by the end of the first month postpartum. These results imply an inhibitory action due to the suckling stimulus, primar-

TABLE XI

MOST ABUNDANT AMINO ACIDS IN NONHUMAN PRIMATE MILK[a]

Amino acids[b]	Rhesus		Long-tailed Baboon		Chimpanzee	
	1–7 days	7 days	(>7 days)	(>7 days)	1–7 days	>7 days
Taurine	60.8 ± 5.6 (37.7)[b]	56.1 ± 5.2 (33.1)	13.5 (21.2)	38.0 ± 4.6 (19.2)	71.0 (29.3)	26.4 ± 1.2 (6.6)
Glutamate	12.3 ± 1.7 (7.6)	31.4 ± 6.9 (18.5)	29.1 (45.8)	43.9 ± 5.6 (22.2)	108.5 (44.7)	264.5 ± 7.5 (65.7)
Serine	12.2 ± 1.2 (7.6)	12.5 ± 3.4 (7.4)	4.3 (6.8)	14.8 ± 2.9 (7.5)	10.2 (4.2)	14.6 ± 1.6 (3.6)
Glycine	9.9 ± 1.7 (6.1)	10.6 ± 2.3 (6.2)	5.0 (7.9)	19.4 ± 3.3 (9.8)	10.4 (4.3)	17.7 ± 2.7 (4.4)

[a]Adapted from Rassin *et al.* (1978). Days refer to days of lactation. Numbers in parentheses indicate percentage of total amino acid pool for each amino acid.
[b]μmoles/100 ml milk ± SEM.

ily on the hypothalmic release of GnRH. Results also suggest that the extension of inhibition of the hypothalamic–hypophyseal apparatus specifically resides in the events surrounding lactation and is unrelated to the endocrine stimulus of pregnancy.

Reports indicate that follicular development may be arrested up through 150 days postpartum (Pope *et al.*, 1986; Weiss *et al.*, 1973; Williams and Hodgen, 1983). Differences in progesterone concentrations for females that do not suckle their young versus those that do show an initial rapid decline after delivery, which has been associated with the collapse of the CL (Goodman and Hodgen, 1964; Plant *et al.*, 1980; Richardson *et al.*, 1985; Weiss *et al.*, 1973). Lactating females indicate much higher levels during the 2 to 3 months following parturition, which has been attributed to further maintenance of the CL during lactation. The continued maintenance of CL function has been attributed to increased prolactin production since the suppression of prolactin or infant weaning results in decreased progesterone production and luteolysis (Plant *et al.*, 1980; Richardson *et al.*, 1985; Schallenberger *et al.*, 1981). However, prolactin does not appear to be directly responsible for the suppression of gonadotropin secretion or the reduction in ovarian activity; the only effects noted after treatment of bonnet macaques with exogenous prolactin were a reduction in the LH response to GnRH and an extension of the period of infertility (Maneckjee *et al.*, 1976).

van der Werff ten Bosch (1982) noted that for the orangutan, cyclicity was resumed approximately 70 days postweaning. Proximity of the infant strongly influenced the duration of amenorrhea, which was directly related to the frequency and intensity of suckling. If the infant died or was removed from the mother, cyclicity resumed within a few weeks or months (Nadler *et al.*, 1981). Both the orangutan and chimpanzee appear to have similar endocrine patterns during lactation (Nadler *et al.*, 1981). FSH is elevated, LH is diminished, and estradiol levels are generally low, whereas progesterone remains at <0.4 ng/ml. The length of amenorrhea has been compared for animals with and without infants (Nadler *et al.*, 1981), and was observed to last for a significantly longer period when infants remained with their mothers. For those animals housed with the dams for 3 to 8 months, the length of anemorrhea was 118 ± 22.7 days whereas amenorrhea in those cases where the infants remained with the dams for up to 1 year was 277 ± 36 days. In contrast, when the newborn was removed within 48 hr after delivery, 370 ± 48 days of amenorrhea was observed. It was noted that a significant feature of the puerperium is the relatively high level of FSH and the low level of LH, which has also been observed in the rhesus (Goodman and Hodgen, 1964). It was also postulated that ovulation inhibition may be related to the change in ratio of these two gonadotropins. Administration of exogenous estrogen to postpartum chimpanzees has been shown to inhibit the ability of the pituitary to secrete FSH in response to GnRH which suggests a selective inhibitory effect of estrogen on FSH. Therefore, the increased levels of FSH during the postpartum period may be the result of low levels of circulating estrogens.

5. Interbirth Intervals

Interbirth intervals consist of three main phases: (1) postpartum amenorrhea, (2) normal cyclicity, and (3) pregnancy. Studies with savannah baboons inhabiting the Amboseli National Park, Kenya, have shown that the presence of an infant reduces the dam's reproductive success by affecting the first and second phase of this cycle (Altmann *et al.*, 1978). Adults will resume cyclicity approximately 3 weeks after the death of an infant, which is significantly different than the usual range of postpartum amenorrhea (mean of 12–12.5 months). This is in direct contrast to data from indoor-housed baboons where a comparable value of 5 to 6 months has been observed. These results suggest that some of the reported differences in baboon populations may be accounted for by the presence of an infant, changes in nutrition, and differences in energy demands. One consequence of rapid conception after loss of an infant is that it enables individuals and populations to recover more rapidly from periods of high infant mortality. Infant loss has a greater impact on shortening interbirth intervals in species with year-round breeding than in those with discrete breeding seasons.

For Japanese macaques, it has been noted that separation of infants from their mothers increases reproductive capability, with optimal time for separation noted to be within 185 days after parturition (Tanaka *et al.*, 1970). Lactation in this species has been reported to last 6 to 7 months in the wild, although 3-month-old infants are capable of eating solid food. With the presumption of 6 to 7 months of lactation, most females will resume normal cyclicity before or during the next mating season.

Interestingly, it has been noted that rhesus females with male infants or infants with increased activity levels tend to conceive sooner than those with female infants or less active infants (Simpson *et al.*, 1981). Those that conceived quickly (defined as a birth in the present season with a second conception in the next mating season) also rejected their infants more frequently at an earlier age, forcing them to develop independence. Analysis of the length of interbirth intervals between successive births for free-ranging *M. sylvanus* indicates 1-year birth intervals as normative and no effect of infant loss on the interval length (Burton and Sawchuk, 1982).

Primiparous females appear to have longer interbirth intervals than multiparous females (Gomendio, 1989). Two hypotheses have been suggested which could explain these differences. First, primiparous macaque females may be less capable of supporting pregnancy due to their diminished size in comparison to fully grown adults. They have achieved only 30% of their adult stature when they enter menarche, which precedes the initiation of the adolescent growth spurt (see Section II). Consequently, they are smaller in size, weigh less, have less body

fat, and are still growing while they raise their infants. This is particularly disadvantageous reproductively, especially when considering the energy demands required for lactation. Second, suckling patterns of primiparous females in comparison to multiparous females suggest alterations in reproductive capacity. Increased suckling bouts are observed with primiparous females which may prevent short-term reproduction. Multiparous females appear to compensate for reduced frequency by increasing duration which appears to reduce the variables that inhibit pregnancy. The reason for increased bouts with shorter duration in primiparous females may be due to limited milk supply which could be the consequence of their limited physical stature and physiologic reserve. The energy requirements for growth may override the production of milk, which results in a small yield.

The nonseasonal baboon shows interbirth intervals of 18 months to 2 years. Prolonged lactation is extended by frequent, short suckling bouts (Hearn, 1984). For feral chimpanzees, interbirth intervals of 5.7 years have been reported; an interval of 3.8 years has been noted for the gorilla (Harcourt *et al.*, 1980). Observations of gorillas living in the wild indicate that lactation has a major influence on birth spacing, with frequency of suckling as an important contributory factor in lactational amenorrhea (Stewart, 1988). The frequency of suckling bouts declines as the infants mature, whereas the duration of bouts remains fairly constant throughout lactation. Low suckling frequencies have been associated with mothers that had resumed cyclicity, whereas higher frequencies were associated with animals that were still acyclic. Results suggested that a frequency of about one bout every 2 hr was critical; those animals with one bout greater than 2 hr remained anovulatory whereas those with one bout less than 2 hr had resumed normal cyclicity.

VII. REPRODUCTIVE SENESCENCE

The information available on aging in nonhuman primates as it relates to reproductive processes is far from complete. Most reports have focused on the macaques (specifically the rhesus and pig-tailed) and the chimpanzee, with more information available for females than males. In the female, reproductive senescence includes the cessation of cyclicity and ovulation, which consists of both a pre- and postmenopausal period. Prior to menopause, ovarian failure is typically characterized by an increase in intermenstrual intervals and a decrease in cycle length. Postmenopausal changes include effects on a variety of organ systems in addition to the reproductive tract (i.e., skeletal, cardiac) which is specifically related to decreased estrogen production. Major characteristics considered as primary features of reproductive senescence in nonhuman primate females include (1) a progressive decline in vaginal bleeding which eventually ceases in some species; (2) changes in patterns of circulating steroid and gonadotropic hormones which is associated with oligo- or amenorrhea; and (3) a decline in reproductive efficiency, with fewer conceptions and increased numbers of stillbirths. For the male, the climacteric is less well-defined. Although a later decline in fertility and sexual activity has been noted, these changes have not been correlated with reductions in circulating androgens.

It should be noted that there are several critical factors that require consideration when evaluating the information available on reproductive longevity. These include normative baseline data on the life span of each species studied (which can be significantly different for animals in the wild versus those in captivity) and the inaccuracies encountered when attempting to assign ages to animals in the wild where birth dates are unknown.

A. Female

1. Prosimians and New World Species

The shortest life spans among primates occur with the prosimian and New World monkeys. Due to the fact that few prosimians are in captivity and many that are wild caught are of unknown age, the numbers of observations are limited. In some species such as the mouse lemur, galago, and loris, and some New World primates such as the tamarin and marmoset, one to two decades represent a reliable life span. The lemurs are the longest lived of the prosimians with a life span of 30–40 years. The slender loris, slow loris, and galagos live from 10 to 15 years and the smaller dwarf lemur and mouse lemur rarely survive a decade. Estimates on tarsiers indicate a 10- to 12-year life span. Little is known of the endocrinological changes in the aging prosimian.

Among the New World species the cebus monkeys live the longest, with some surviving to 40–45 years of age. Spider monkeys live 30–35 years and many other species live 10–20 years. It is anticipated that the average life span will increase with better conditions of nutrition and medicine (Bowden and Jones, 1979). Although many of the New World primates will continue breeding until old age, the frequency may reduce markedly after 10 years. There is no evidence, to date, for the existence of menopause in the New World species.

2. Old World Species and the Great Apes

Of the macaques, the rhesus has been the most extensively studied. Reports by van Wagenen (1972) have indicated that the climacteric for this species occurs at 25–30 years of age as evidenced by a decline in cyclical regularity. A characteristic postmenopausal appearance in 30-year-old females was described which includes facial pigmentation and gray/thinning hair. Collins *et al.* (1983) recorded perimenopausal changes in the ovaries beginning at ~22–25 years of age with a rapid

decline in folliculogenesis thereafter. Ovulatory function appeared to cease at ~30–31 years. Hodgen *et al.* (1977a) studied the hormonal and menstrual patterns of 17 females ≥22 years and found that sustained elevations of the gonadotropins and low estradiol and progesterone concentrations were associated with oligo-/amenorrhea. Menstrual flow was observed from 0 to 16 times over a 15-month period. Five animals failed to show any sign of menses, 8 animals exhibited ≤ five episodes of vaginal bleeding, and the intermenstrual intervals were highly variable, ranging from 11 to 157 days. In the remaining 4 animals, vaginal bleeding was detected 10–16 times at nearly regular intervals. During this transitory phase, FSH appeared to be modulated independently of LH. The gonadotropins remained three to five times higher than basal levels in normal cycling animals and circulating estradiol and progesterone never rose above 100 pg/ml and 1 μg/ml, respectively. It was concluded that menopause occurred in the rhesus during the third decade as vaginal bleeding declined and eventually ceased in all females studied. Further hormonal data were provided by Williams and Hodgen (1982) who reported that the secretion of LH and FSH was exaggerated during the final cycle, particularly at the time of the midcycle surge. Eventually, basal levels of the gonadotropins increased to castrate concentrations with FSH ascending prior to LH. Data also supported prior observations regarding the decline in vaginal bleeding with ultimate cessation during the third decade (reproductive life span from 5 to 25 years of age). Hormonal patterns for *M. silenus* are similar to *M. mulatta*; parallel studies identified three consistent patterns seen in "elderly" animals which provide a means for the characterization of menstrual status and reproductive potential (Cranfield *et al.*, 1988). These include (1) rising estradiol and progesterone (≥2 ng/ml) with normal FSH and LH indicative of ovulatory cycles; (2) low estradiol and progesterone in conjunction with FSH and LH levels which never rise above midcycle levels indicative of secondary amenorrhea (due to hypothalamic dysfunction); and (3) continuously low estradiol and progesterone with elevated FSH and LH above the midcycle surge indicative of menopause.

Lapin *et al.* (1979) observed a somewhat earlier period ("early twenties") for menstrual irregularities and the occurrence of menopause which was associated with morphological changes including bilateral ovarian cysts, uterine atrophy and sclerosis, and cervical polyps. It was also noted that conceptions declined in the older females (>13–17 years). Amenorrhea, follicular depletion, absence of corpora lutea, and cortical fibrosis of the ovaries were noted in two females >23 years of age in an earlier study (van Wagenen and Simpson, 1965), suggesting that there is insufficient follicular tissue for normal gonadal steroidogenesis. Evaluation of 17 female pig-tailed macaques (*M. nemestrina*) both hormonally and anatomically (4 to ≥20 years of age) revealed little difference between 10- and 20-year-old animals in mean weight of the reproductive organs (Graham *et al.*, 1979), although none of the 20 year olds showed an abundance of ovarian follicles typical of 10 year olds. One 20-year-old female had all the hormonal and morphological indications of menopause, including amenorrhea; increased LH; gonadotrope hypertrophy; a low estrone:estradiol ratio; ovarian changes such as decreased weight, follicular depletion, absence of corpora lutea, and fibrosis; and atrophy of the uterus and vagina. Although females ≥20 years maintained cyclicity, ovarian changes were observed such as decreased follicular content, cortical fibrosis, involuted uteri, cervical atrophy, and increased ceroid associated with regressing luteal bodies.

Small (1984) reported that although older female rhesus evaluated in a group of outdoor-housed animals experienced a decrease in fertility (age range of 2–17 years), few reached true menopause. The total infant loss rate (abortions and stillbirths) over a 6-year period was 15.8%, which is comparable to rates reported for both laboratory (Binkerd *et al.*, 1988) and feral groups of macaques (Small, 1982). Similar findings were reported by Wilson *et al.* (1978) who observed that the older females in a group of 71 animals experienced more nonreproductive years in addition to an increase in infant loss. An increase in the percentage of stillbirths has been noted for *M. nemestrina* females >15 years of age (from <1 to 50%) (Graham *et al.*, 1979). In addition, preliminary studies with bonnet monkeys (22 females 11.5–16.5 years of age) indicated reduced reproductivity (pregnancy rate of 22%) with only 50% infant survival (Jensen *et al.*, 1980). It was also noted that older monkeys were more vulnerable to stress since a high mortality rate was associated with illness. Contrary to these findings, observations by Silk *et al.* (1981) did not provide evidence for reproductive cessation in female bonnets up to 15–16 years of age, although Jensen *et al.* (1982) had noted a decrease in sexual interaction in this species from 16 to 17 years ($N=7$; perimenopausal). Diminished estradiol concentrations at midcycle were also reported. A gradual decline in sexual responsivity or arousal may occur in pig-tailed females (17–18 years of age) which may precede endocrine changes but has not been shown to be reversed with exogenous hormonal supplementation (Sassenrath *et al.*, 1982). Data also suggest that the endocrine status of the female may be critical in altering the behavioral response of the male.

Of interest are studies with *M. nemestrina* (Short *et al.*, 1987, 1989) where variables indicative of aging were assessed. An evaluation of 72 variables in 30 females (age groups included 8–9, 12–17, and 18–28 years of age) isolated 28 significant variables which were found to be influenced by age or by age in conjunction with diet or gender (Short *et al.*, 1987). These included body temperature, blood albumin, bone thickness, and hemoglobin concentrations. Further studies with 27 females (9–31 years of age) showed that ovarian cyclicity diminished at ~18 years, although some animals maintained normal cyclicity up through 20 years of age (Short *et al.*, 1989). Some females displayed a decreased cycle intensity, whereas others showed a deterioration in periodicity with missed cycles and detumescence or lengthy cycles with tumescence for up to 2 to 3

months. Similar to findings in rhesus females, lower estradiol concentrations and higher levels of FSH and LH were observed in older individuals.

The activities of feral groups of *M. fuscata* have been observed (Takahata, 1980; Wolfe and Sabra Noyes, 1981), particularly the Arashiyama troop of Japan. Of the 388 recorded births to females of known age, decreased birth rates were noted in the older females with no births recorded for females >22 years of age. In addition, it was noted that the maximum life span for this species was 27 years. Females ≥17.5 years showed low sexual activity and decreased conception rates (Takahata, 1980). All animals within the 20.5–25.5 age range continued to cycle. which led to the conclusion that menopause occurred at 25–26 years of age. Wolfe and Sabra Noyes (1981) studied the reproductive potential of 28 Arashiyama females ≥18 years of age and found that fertility usually ended at 22 years despite one unexpected birth in a 25-year-old female. Data suggest that the decline in fertility was not due to menopause since aging females did continue to cycle, although the cycle duration was considerably shorter when compared to younger animals. During the transport of this troop from Japan to Texas, the reproductive capabilities of the older females were less affected than the younger females, although a brief communication by Gouzoules *et al.* (1984) suggests that a data error may have occurred which, once corrected, indicated no differences for birth rates in the older and younger females.

Other macaques have been monitored under feral conditions, although data are limited. These include *M. arctoides* (decreased reproductive capacity, increased interbirth intervals, and stillbirths with advancing age) (Harvey and Rhine, 1983) and *M. sinica* (fertility significantly reduced in females in their mid-twenties) (Dittus, 1977).

Less data are available for baboons and the great apes. *P. anubis* females have been observed in the wild (Strum and Western, 1982), and aging criteria have been established based on physical appearance. It was deduced that while interbirth intervals increased, fecundity decreased with advancing age. For free-ranging *P. hamadryas*, one older female (age unknown) was noted with menstrual irregularities and eventual cessation of cyclicity (Sigg *et al.*, 1982).

One of the more comprehensive studies of the chimpanzee indicates that of the 10 females ranging in age from 35 to 48 years, most continued cycling until their death (Graham, 1979). All had at least one cycle the last year of their life, and cycle lengths continued to increase with advancing age for some. The conception rate was significantly reduced when compared to 15- to 25-year-old females (3.9% versus 20%), although it should be noted that the animals were bred less frequently. Three conceptions occurred in females ≥35 years; only one 38-year-old female produced a viable offspring. The oldest recorded conception occurred at 40 years of age. Physical evidence of aging included skin wrinkling, joint stiffness, muscle atrophy, advanced atherosclerosis, and pigment deposition (hemosiderin). Histologically, ovaries showed follicular depletion and advanced sclerosis of the vessels which was characterized by subintimal and medial fibrosis resulting in a thickening of arteriolar walls and a reduction in lumen size. One animal had a uterine leiomyoma, three had adenomyosis, and one displayed endometrial hyperplasia. Based on these findings, it was concluded that none of the animals exhibited menopause (i.e., cessation of cyclicity and atrophy of the reproductive tract), although evidence of impending senescence was present.

Additional studies further supported these findings (Gould *et al.*, 1981). Two females ≥40 years of age were compared to 51 females 18–39 years of age. An increase in cycle length and a decrease in menstrual frequency were observed. In addition, estrone and pregnanediol glucuronide were evaluated and concentrations differed only slightly between the two groups. One aged female showed reduced estrogens but normal gonadotropin levels. An anecdotal report regarding one female pygmy chimpanzee (*P. paniscus*) ≥40 years of age indicated cessation of cyclicity and elevation of gonadotropins with a reversed FSH:LH ratio (Gould *et al.*, 1981). It was concluded that a slow reduction in the frequency of menses during the fifth decade with abrupt cessation may be characteristic of this species, which is unlike the human female.

The existing data suggest that, in the macaque species studied to date, menopause occurs in some but not in all animals and that a great variability in age regarding when cycling ceases exists (Small, 1984); the factors that contribute to the climacteric, when it does occur, are unknown. The failure to observe this condition in the chimpanzee suggests that the regular occurrence of menopause may be limited to the human female.

B. Male

There has been little systematic research regarding the study of reproductive aging in male nonhuman primates (Graham, 1986). This lack of intense study probably reflects the slow progressive decline in reproductive activity in the males. The aging phenomena are important in captive colonies where a decline in fertility with age is an important aspect of breeding management (see Chapter 14).

1. Prosimians and New World Species

No studies have been reported on the reproductive senescence in male prosimians or New World primates.

2. Old World Species and the Great Apes

Robinson *et al.* (1975) noted that sexual vigor and ejaculation frequency declined in rhesus males over time when compared to younger or middle-aged males, although there was no difference between age groups in levels of testosterone. The lack of correlation between decreased reproductive potential and

circulating testosterone has been recorded in several other studies with this species (Bremner *et al.*, 1983; Chambers and Phoenix, 1981; Chambers *et al.*, 1981; Phoenix and Chambers, 1984). No differences were observed between groups of 18 males (9 at ~10 years of age, 9 at ≥20 years of age) for diurnal variations in testosterone, DHT, cortisol, or estradiol (Chambers and Phoenix, 1981). Further studies attempted to show that alterations in CNS availability of testosterone was a critical factor in the behavioral changes noted in the older males rather than absolute levels of circulating testosterone. No difference in the mean level of total testosterone or index of free testosterone was apparent, although older males had a significantly higher percentage of testosterone bound to testosterone-binding globulin (66% versus 57%) (Chambers *et al.*, 1981). However, no change in binding activity was noted. Neither total serum testosterone nor the index of free testosterone correlated with the level of sexual performance. Two additional studies with this species (16 males: 8 at ~12 years of age, 8 at ~22 years of age; 18 males: 9 at ~8–14 years and 9 at ~18–24 years) (Phoenix and Chambers, 1984) showed no differences in testosterone, LH, or sexual activity with increasing age or with relocation. It was concluded that, based on these data, the male macaque undergoes a decline in reproductive behavior which involves complex changes in the CNS instead of a reduction in circulating steroid hormones.

Lapin *et al.* (1979) reported that the number of conceptions from a male hamadryas baboon declined precipitously from the mid-twenties to age thirty. Morphological changes associated with aging in feral male Chacma baboons (*P. ursinus*) (10 animals >15 years of age) included penile angiopathy with lumen stenosis (intima thickening due to intimal proliferation and fibrosis), an increase in the fibrous tissue content of the endothelium (fibrous trabeculopathy), and intratrabecular senechiae (Bornman *et al.*, 1985). It was concluded that the baboon could serve as a model to study impotence related to vascular changes in aging males.

The limited availability of aging nonhuman primates of known age has hampered major advances in research. In the event that larger numbers of animals become available in which both invasive and noninvasive procedures can be practiced, the new technologies currently being developed should contribute to a better understanding of the aging process.

VIII. MALE

As with other areas of primate reproduction discussed in this chapter, the majority of information available regarding the male has come from studies performed with macaques, specifically the rhesus. Excellent reviews on the endocrine regulation of male reproduction have been published (Graham, 1981a; Wickings *et al.*, 1986), therefore, only a summary of the principal mechanisms which control these functions and the complex interactions regulating the hypothalamic–pituitary–testicular axis will be presented here.

The control of spermatogenesis in nonhuman primates involves a variety of factors, some of which are not well understood. These include the pulsatile secretion of GnRH (also referred to as luteinizing hormone-releasing hormone), stimulation of the gonadotropes of the anterior pituitary with subsequent secretion of LH and FSH, production of testosterone by the Leydig cells of the testis, and a feedback interaction of testosterone with LH/FSH. Although LH appears to maintain Leydig cell function and spermatogenesis in most mammalian species, FSH may play a central role in the control of spermatogenesis in primates.

The lack of extensive data on male reproductive function not only reflects the numbers of available animals for analysis but also the problems involved in measuring hormone output. Developing sensitive assays for the gonadotropins is particularly problematic, especially for measuring circulating gonadotropins such as FSH. In addition, FSH levels in the male are considerably lower than in females, which further complicates accurate assessments of concentrations or interactions.

Some species exhibit annual fluctuations in gonadal function, with gametogenesis confined to specific breeding seasons. It has been shown experimentally that alterations in the ability of the testis to bind gonadotropins is one possible mechanism involved in this effect (Bartke *et al.*, 1987). Although it was assumed that the female initiated the seasonal cycle of testicular function (Conaway and Sade, 1965; Sade, 1964; Zamboni *et al.*, 1974), this has been refuted (Gordon *et al.*, 1976; Michael and Keverne, 1971; Robinson *et al.*, 1975; Wickings and Nieschlag, 1980a).

The basic morphology of spermatozoa in nonhuman primates is similar, although species differences do exist. Generally speaking, sperm consists of four basic components: (1) the *head* which contains chromatin and is capped by the acrosome; (2) the *neck* which contains the basal plate, connecting pieces, and a centriole; (3) the *midpiece* which contains the mitochondria; and (4) the *tail*.

Both LH and FSH are necessary for normal spermatogenesis (see Section VIII,B,3). Three general processes are involved in the development of spermatozoa: (1) mitotic division by which spermatogonia either renew and proliferate or produce spermatocytes; (2) meiotic division of the diploid primary spermatocytes which results in haploid spermatids; and (3) spermiogenesis, which is the process by which spermatids become spermatozoa (Clermont, 1972; Wickings *et al.*, 1986). The seminiferous tubules within the testis are comprised of all of these cell types—spermatogonia, primary and secondary spermatocytes, spermatids, and spermatozoa—which progress from the outer surface (less mature form) to the lumen (more mature form). The seminiferous tubules and the supportive, or Sertoli, cells constitute the seminiferous epithelium.

A. Prosimians and New World Species

1. Spermatogenesis

A number of comparative studies on the morphology of primate spermatozoa utilizing scanning electron microscopy have been published (Bedford, 1967; Gould and Martin, 1978; Matano *et al.*, 1975) which include details of the ultrastructural (and identifying) features of the greater and lesser galago; ringtailed, black, and ruffed lemurs; and slow loris. The sperm head of the bushbaby (*G. senegalensis*) is complex and has features in common with several other lower forms such as a system of membranes which cover the neck and anterior midpiece. The sperm of lemurs vary considerably—*Hapalemur griseus* and *L. variegatus* are elliptical in shape and the tail inserts eccentrically into the posterior head surface. *L. macaco* and *L. catta* have a more rounded, paddle-shaped head, and are uniformly flattened dorsoventrally (Gould, 1980; Harrison and Lewis, 1986). In the Platyrrhine, the midpiece often inserts eccentrically into the posterior border of the head. The posterior acrosomal margin of squirrel monkey sperm has a serrated appearance and a smaller size when compared to the capuchin, which have typical paddle-shaped sperm heads (Barr, 1973). Sperm parameters have been assessed in a number of New World species (Bush *et al.*, 1975; Gould and Martin, 1978; Harrison and Wolf, 1985).

Sperm maturation in the epididymis has been studied in the marmoset (Moore, 1981; Moore *et al.*, 1984); a pattern similar to that observed in other primates has been noted.

2. Seminal Fluids

No studies have evaluated capacitation or measured the biochemical parameters of sperm or plasma fractions of the semen in prosimians.

3. Hormonal Regulation

The male squirrel monkey (*Saimiri boliviensis boliviensis*) has a well-defined breeding season (see Section VIII,A,4) characterized by low levels of DHEA when compared to circulating levels of testosterone and androstenedione (Wiebe *et al.*, 1984). In contrast to humans and macaques, androstenedione is the major androgen produced by this species during the breeding season. Mean androstenedione concentrations have been reported to increase from nonbreeding season levels of 91.4 ± 12.9 ng/ml to prebreeding concentrations of 139.0 ± 10.5 ng/ml and a breeding season peak of 167.5 ± 15.4 ng/ml (Wiebe *et al.*, 1988). Although individual serum androgen concentrations fluctuate considerably throughout the year, a significant seasonal variation in DHEA, testosterone, and androstenedione does occur (Wiebe *et al.*, 1988) (see Table XII).

Little information is available on the reproductive physiology of the male cebus monkey. Plasma testosterone levels range from 5 to 110 ng/ml with levels ~seven-fold greater than those reported for Old World species (Nagle and Denari, 1982, 1983). No consistent pattern of androgen levels have been shown either within or between animals throughout the year.

4. Seasonality

Seasonal responses have been noted in male reproductive capabilities, but the exact environmental cues are not clearly evident. A number of prosimians, including the ruffed and mongoose lemurs, show a testicular volume increase as the breeding season approaches with maximum size obtained about 1 month prior to the initiation of breeding. This volume increase has been calculated to be over 150%; a body weight increase of about 14% is also observed during this period.

In male squirrel monkeys, once adult testosterone levels are achieved, the seasonal pattern becomes well-established. Males will show progressive stepwise increments in their annual testosterone peaks for several years, which has a pronounced effect on their body weights and is positively correlated with both cortisol and testosterone concentrations. Seasonal changes have been well-documented in this species and have been reviewed by Lindburg (1987). Adult males undergo an annual weight gain of roughly 200–300 g ("fatted male response") which begins 2 to 3 months before mating activity (DuMond, 1968). At the time of the weight gain there is also an increase in dominance and aggressiveness, even in the absence of females. Undoubtedly the circulating androgens (testosterone androstenedione, DHEA) all play a role in this effect with some influence on behavior (Wiebe *et al.*, 1988). Other factors, however, are also pertinent. For example, animals housed in breeding colonies show seasonal variations except when caged individually or when access to females is restricted. Under these conditions, they do not show any circannual variation in plasma androgen levels although large fluctuations (including diurnal variations) are evident (Wiebe *et al.*, 1984).

The marmoset does not show the pronounced fatting syndrome observed in squirrel monkeys, but testicular steroidogenesis has shown a distinct diurnal pattern which is characterized by low androgen levels in the daylight (Kholkute, 1984; Preslock and Steinberger, 1977). Prolactin levels are also increased in the male particularly when they participate in infant-rearing activities, although this is not reproductively suppressive.

In nocturnal owl monkeys, levels of plasma testosterone show a diurnal rhythm with the highest levels occurring during darkness (24.8 ng/ml) (Dixson and Gardner, 1981). Spermatogenesis appears to be partially arrested in this species throughout the year. Semen samples collected by electroejaculation indicate very low sperm numbers and, based on histological studies, few sperm are present with many spermatocytes degenerated and deposition of orange-brown pigment present in the intertubule tissues (Dixson, 1983). Despite this unique characteristic, fertility is maintained as evidenced by regular conception

TABLE XII

MALE REPRODUCTIVE PARAMETERS[a]

Species	Spermatogenesis (days)	Ejaculate (semen) volume (ml)	Sperm			Plasma testosterone (ng/ml)
			Concentration ($\times 10^6$/ml)	% motility	% normal	
S. sciureus	39	0.2–1.5	80.8–310.9 (205.9)	52 (40–80)	—	52.2 ± 11.6 [June–Sept.]
						103.5 ± 12.8 [Dec.–March]
C. aethiops		0.3–2.0 (0.9)	165.8–810.8 (439.6)	39 (15–70)		8–20
M. mulatta	36	0.4 ± 0.06	618 ± 125 (N=23) [Jan]	57 ± 6[b]	71 ± 5	5.5 ± 0.5 nm/l [March–May]
			758 ± 220[b] (N=18) [March]	51 ± 7[b]		8.9 ± 2.0[b] [Aug.–Sept.]
			381 ± 133[b] (N=7) [May]	43 ± 13[b]		23.5 ± 5.8[b] [Oct.]
			441 ± 217[b] (N=7) [July]	53 ± 13[b]		
			439 ± 215[b] (N=6) [Sept.]	57 ± 9[b]		
			348 ± 127[b] (N=14) [Nov.]	61 ± 6[b]		
M. fascicularis	36	0.26 ± 0.03	1638 ± 115	84 ± 1	77 ± 3	16.7 ± 1.1 [Sept.–Oct.]
						9.5 ± 0.9 [March–June]
M. arctoides	36	—	—	—	—	14.8 [June]
						6.7–7.2 [Aug.–Sept.]
M. fuscata	36	—	—	—	—	7–13
M. nemestrina	36	—	—	—	—	9–16.5
M. radiata	36	2.2 ± 0.2 [winter]	1251 ± 165	72 ± 2		21.3 ± 4.1
		1.9 ± 0.2 [summer]	1195 ± 145	74 ± 2		
P. ursinus	42	—	—	—		18.3 ± 3.2
P. troglodytes	—	0.1–2.5 (1.1)	548 (54–2750)	30 (10–60)		409 ± 45
P. pygmaeus	—	0.2–3.6	76 (10–165)			1003 (628–1421)
		0.2–3.2	10–128	50	60	2367
G. gorilla	—	0.38	41	32	49	413.7 ± 219.1

[a]From Dang and Meusy-Dessolle (1981); Graham (1981a); Harrison and Lewis (1986); Wickings *et al.* (1986); and Wiebe *et al.* (1988).
[b]SEM

rates, even with the infrequent copulatory activity noted in family groups.

B. Old World Species and the Great Apes

1. Spermatogenesis

Sperm from various Cercopithecidae species appear uniform in shape, particularly in comparison to the great apes (Harrison and Lewis, 1986). The sperm heads appear flat and paddle-shaped, with the midpiece long in relation to the head, and the mitochondria of the midpiece small and well-organized (Harrison and Lewis, 1986), whereas the sperm heads of the baboon are short, oval, paddle-shaped, and taper anteriorly (Flechon *et al.*, 1976). The anterior segment of the acrosome in this species is surrounded by a marginal thickening and covers roughly two-thirds of the head. The midpiece is characterized by a relatively long and regular helical sheath of mitochondria, with the ends of the mitochondria randomly distributed. Morphology and dimensions have been compared to other nonhuman primates and are very similar to those of Cercopithecidae (Flechon *et al.*, 1976).

Gould (1980) has provided an excellent description of spermatozoa for a variety of the great apes. Orangutan sperm have a relatively large, flat, and paddle-shaped head and a tail which is attached centrally to the posterior border of the head with the anterior surface primarily covered by the acrosomal cap. The sperm of these species are relatively uniform in contrast to those of the gorilla, which are more pleomorphic. Sperm heads of the gorilla are small and thickened posteriorly, and thinned or hollowed anteriorly. The midpiece is short, disorganized, and contains few mitochondria. Of the great apes, the spermatozoa of the chimpanzee (*P. troglodytes* and *P. paniscus*) are the most uniform. The sperm heads are relatively small and thickened posteriorly and the midpiece is similar to that of the gorilla.

Detailed investigations of spermatogenesis are limited to the rhesus (Arsenieva *et al.*, 1961; Barr, 1973; Clermont and LeBlond, 1959; Conaway and Sade, 1965; de Rooij *et al.*, 1986; Fawcett *et al.*, 1970), the long-tailed (Dang, 1970; Fouquet and Dadoune, 1986; Kluin *et al.*, 1983) and stump-tailed macaque (Clermont and Antar, 1973), the green monkey (Clermont, 1969), *C. sabolus* (Barr, 1973), and the olive (Afzelius *et al.*, 1982; Chowdhury and Marshall, 1980; Chowdhury and Steinberger, 1976) and yellow baboon (Barr, 1973). Clermont and LeBlond (1959) described 12 stages in the cycle of the rhesus

TABLE XIII

BIOCHEMICAL PARAMETERS OF SERUM AND TESTICULAR FLUIDS FROM RHESUS MONKEYS[a]

Parameter	Testicular fluid	Serum
pH	7.2–7.4	7.4–7.6
Total protein (g/100 ml)	4.63	1.27
Lactate dehydrogenase (units/mg protein/min)	0.15	0.005
Glucose-6-phosphate dehydrogenase		
Racker units/100 ml	4.0	2.3
units/ml	6533	0.0
Acid phosphatase (mg P/100 ml/hr)	91.4	1.8
Alkaline phosphatase (mg P/100 ml/hr)	29.1	44.7
Hyaluronidase (units/100 ml)	0.0	0.0
Glucose (mg/100 ml)	30.4	131.7
Glycogen (mg/100 ml)	26.3	6.5
Lactic acid (mg/100 ml)	579.0	92.0
Ascorbic acid (mg/100 ml)	9.93	0.34
Total lipids (mg/100 ml)	141.55	453.67
Phospholipids (mg/100 ml)	18.37	157.53
Sodium (mEq/liter)	191.29	179.57
Potassium (mEq/liter)	8.18	5.63
Calcium (mEq/liter)	3.13	6.85
Chloride (mEq/liter)	127.62	114.32

[a]Adapted from Pande *et al.* (1969) and Harrison and Lewis (1986).

seminiferous epithelium. Steps were defined by changes in the nucleus and acrosomal structures, and it was noted that each stage appeared in sequence with time over a particular area in a given seminiferous tubule. Tubule cycle durations have been reported to range from 9 to 12 days and, depending on the species, the total duration of spermatogenesis has been noted to range from 36 to 48 days (Table XII). Due to the haphazard arrangement of some of the cellular stages within the seminiferous tubules, *P. anubis* appears to be more intermediate between the human and other primates in its development of spermatozoa (Wickings *et al.*, 1986). However, the overall similarity observed between these species in cell type, association, and kinetics suggests a similar hormonal control.

Capacitation is a phenomenon affecting the sperm which normally occurs within the female reproductive tract and, through enzymatic action, renders the sperm capable of fertilization (Dukelow and Yorozu, 1986). In most primate species studied, this requires approximately 2 to 8 hr.

2. Seminal Fluids

Information on the biochemical parameters associated with testicular and epididymal fluids is limited, with the most data available for the rhesus. Although some species differences have been noted, these differences may be dependent on the methods used for analyses (Harrison and Lewis, 1986). Inasmuch as fluids vary in the different regions of the male reproductive tract due to absorptive and secretive mechanisms, there are significant differences between blood and reproductive tract fluids, which, in many cases, are due to the presence of the blood–testis barrier (White, 1981). For example, testicular fluid in the rhesus consists of greater volumes of lactate dehydrogenase, glucose-6-phosphate dehydrogenase, lactic acid, and ascorbic acid than does serum, and the converse is true for glucose and total lipids (Tables XIII and XIV).

The primary functions of the epididymis are maturation and storage of spermatozoa. The fluid of the epididymis contains a variety of compounds derived from rete testicular fluid, which is modified by the epididymal epithelium. Testicular fluid has low concentrations of spermatozoa and is characterized by a low glucose and high inositol content (White, 1981). Collection of fluid from the cauda epididymis indicates that the composition is similar among species, although slight variations are noted. The chief characteristics of the cauda epididymal plasma are low concentrations of inorganic ions and high levels of organic constituents such as glycerylphosphylcholine, carnitine, sialic acid, hypotaurine, glycosidases, and phosphatases. The

TABLE XIV

BIOCHEMICAL PARAMETERS IN EPIDIDYMAL FLUIDS FROM RHESUS MONKEYS[a]

Parameter	Caput	Corpus	Cauda
pH	6.70 *(1)*	6.70 *(1)*	7.00
Total protein (g/100 ml)[b]	141.10 *(3)*	0.95 *(4)*	7.40
Acid phosphatase (mg P/100 ml/hr)	1.67 *(1)*	0.52 *(1)*	0.36
Alkaline phosphatase (mg P/100 ml/hr)	5.06 *(1)*	8.14 *(1)*	10.32
Hyaluronidase	133.00 *(1)*	165.00 *(1)*	173.00
Glycogen (mg/100 g)	38.40 *(3)*	31.6 *(3)*	41.00
Total lipids (mg/10^8 cells)	15.73 *(4)*	2.09 *(4)*	0.56
Phospholipids (mg/10^8 cells)[c]	25.13 *(3)*	14.02 *(3)*	16.50
	3.52 *(4)*	0.45 *(4)*	0.13
Lactic acid (mg/g)	3.48 *(3)*	3.20 *(3)*	3.41
Sialic acid (mg/g)	51.06 *(6)*	48.60 *(6)*	37.10
GPC (mg/g)[d]	4.1 *(3)*	4.45 *(3)*	7.32
			2396.00
Sodium (mg/g)	3.09 *(1)*		3.42
			18.20
Potassium (mg/g)	2.35 *(1)*		1.69
			49.20
Chloride (mg/g)	2.68 *(1)*		3.42
			10.50
Total phosphorus (mg/100 ml)			238.00
Inorganic phosphorus (mg/100 ml)			5.20
Acid-soluble phosphorus (mg/100 ml)			230.00
Lactate dehydrogenase (IU)			1845.00

[a]Adapted from *(1)* Riar *et al.* (1973b); *(2)* Jones (1978); *(3)* Riar *et al.* (1973a); *(4)* Arora *et al.* (1975); *(5)* Gupta and Dixit (1981); *(6)* Bose and Kar (1968); and *(7)* Harrison and Lewis (1986).

[b]Protein values for ref. *(4)* are expressed as mg/10^8 cells.

[c]Phospholipid values for ref. *(3)* are expressed as mg/g.

[d]GPC values for ref. *(2)* are expressed as mg/100 ml.

TABLE XV

BIOCHEMICAL PARAMETERS IN SPERM AND PLASMA FRACTIONS OF SEMEN FROM NONHUMAN PRIMATES[a]

	Lactic acid		Citric acid		Fructose	
Species	SF	PF	SF	PF	SF	PF
C. apella	0	4	13 ± 19	79 ± 85	0	563 ± 496
S. sciureus	42	151	3 ± 4	48 ± 14	0.4 ± 1	110 ± 129
E. patas	34 ± 21	194 ± 222	31 ± 52	127 ± 62	18 ± 39	315 ± 274
C. aethiops	20 ± 12	192 ± 196	5 ± 10	122 ± 25	10 ± 16	264 ± 175
M. mulatta	32 ± 30	138 ± 115	2 ± 5	157 ± 86	14 ± 28	753 ± 900
M. fascicularis	36 ± 17	239 ± 88	0	101 ± 65	7 ± 13	299 ± 264
M. arctoides	28 ± 30	183 ± 186	6 ± 9	231 ± 121	0	262 ± 108
T. gelada	10	130	16	168	0	160
P. troglodytes	24 ± 16	160 ± 127	15 ± 24	256 ± 191	10 ± 30	497 ± 363

[a]Data adapted from Ackerman and Roussel (1968) and Harrison and Lewis (1986). Sperm fraction (SF): all parameters expressed in mg/100 ml. Plasma fraction (PF): all parameters expressed in mg/100 ml.

concentrations of sodium ions are ~20 mEq/liter. The potassium ion levels are generally greater than or equal to sodium, although, in the rhesus, potassium is twice the sodium concentration. It is generally accepted that the pH varies along the length of the epididymis, but is usually within the 6.5–7.0 range, although it may occasionally be slightly higher (White, 1981). The composition of the epididymal fluids has been reported for the rhesus monkey (Arora *et al.*, 1975; Bose and Kar, 1968; Jones, 1978; Riar *et al.*, 1973a,b) and, to a more limited extent, for the langur (Gupta and Dixit, 1981). Ackerman and Roussel (1968) conducted one of the few comparative studies reported for nonhuman primates. Table XV lists three biochemical parameters, namely, lactic acid, citric acid, and fructose, found in the semen of 10 nonhuman primate species. Although our current understanding of the functional aspects of these fluids is limited, state-of-the-art microanalytical and *in vitro* techniques should improve methods for analysis, thereby increasing our knowledge (Hinton, 1980; Hinton and Howards, 1982).

The volume and concentration for L-carnitine, fructose, citrate, and acid phosphatase for the liquefied fraction and the coagulum have been compared in semen obtained from chimpanzees (Marson *et al.*, 1989). A close correlation between L-carnitine and the sperm count suggests that this component is primarily a product of the epididymis. Correlations were also noted between total concentrations of L-carnitine, citrate, and acid phosphatase; fructose and acid phosphatase were considered to be specific markers of the seminal vesicles and the prostate, respectively, as noted previously (Martin and Gould, 1981). Citrate was also closely correlated with acid phosphatase but not with fructose, suggesting a prostatic origin in this species. It was also observed that the percentage of liquefaction of the ejaculate was positively correlated with citrate and acid phosphatase concentrations (of prostatic origin) but negatively correlated with concentrations of fructose (from seminal vesicles). These results suggest that, in this species, the coagulation–liquefaction process depends on a balance between seminal and prostatic secretions.

The rhesus ejaculate has been noted to solidify immediately upon expulsion. Further information regarding methods for obtaining ejaculates and their characteristics is described in Chapter 14.

3. Hormonal Regulation

Once sensitive radioimmunoassays (Robinson *et al.*, 1975) and *in vitro* bioassay systems were developed (Steiner *et al.*, 1980; Wickings *et al.*, 1979), it was demonstrated that GnRH stimulates LH release from the anterior pituitary in the male rhesus (Ferin *et al.*, 1974; Toivola *et al.*, 1978; Wickings and Nieschlag, 1980a), the long-tailed monkey (Mori and Hafez, 1973), and the baboon (Koyama, 1976). Further evidence for the stimulatory effect of GnRH on LH secretion was provided by studies where the GnRH stimulus was abolished by transection of the hypothalamic stalk (Marshall *et al.*, 1983), by active immunization against GnRH (Chappel *et al.*, 1980; Hodges and Hearn, 1977), or by administration of GnRH antagonists (Weinbauer *et al.*, 1987, 1989; Weinbauer and Nieschlag, 1989). Results of transection or immunization indicate a decrease in circulating levels of LH and testosterone, which was followed by testicular atrophy, whereas exposure to GnRH antagonists resulted in a decreased testicular size, changes in testicular morphology, and suppression of sperm counts.

LH stimulation of the Leydig cells within the testis is responsible for maintaining high concentrations of testosterone, the androgen essential for spermatogenesis. The mechanism by which LH controls androgen secretion has been studied (Arslan *et al.*, 1986), and it was noted that chronic gonadotropin exposure (hCG) resulted in the activation of the stimulatory response required for testosterone production. It was proposed that this occurred via enhancement of LH/CG receptor availability. The connection between LH stimulation and testicular steroidogenic activity appears to be the LH receptor on the Leydig cell membrane (Wickings *et al.*, 1986). Reports indicate that only partial receptor occupancy is necessary for the stimulation of testosterone production. The testis of the rhesus monkey responds rapidly to LH; increased levels of serum testosterone are evident within 30 min with maximum concentrations achieved by 1 hr (Toivola *et al.*, 1978; Wickings and Nieschlag, 1980c). Administration of hCG for a 3-day duration (5,000 IU/day) results in a 10-fold increase in serum testosterone levels in the adult rhesus (Wickings *et al.*, 1986), although it should be noted that hCG is an antigenic stimulus and antibody production will occur after repeated administration (Catchpole and van Wagenen, 1975; Nieschlag and Wickings, 1980). Further studies have shown that daily treatment of adult long-tailed monkeys with 450 IU hCG for 16 days will result in a 163% increase in the

number of Leydig cells and a 9-fold rise in plasma testosterone concentrations (Teerds *et al.*, 1989).

Leydig cell function varies according to both internal and external factors, which results in diurnal variations in serum testosterone. The highest levels are observed at night, with changes noted as great as 100% in the rhesus (Goodman *et al.*, 1974; Michael *et al.*, 1974; Perachio *et al.*, 1977; Plant, 1980). Diurnal patterns of testosterone secretion have also been shown in the bonnet, long-tailed, and African green monkey in addition to the baboon (Beattie and Bullock, 1978; Bielert and Vandenbergh, 1981; Mukku *et al.*, 1981; Steiner *et al.*, 1980). These nocturnal elevations have been attributed to an increase in the frequency and amplitude of LH pulses (Plant, 1980; Steiner *et al.*, 1980). Exogenous factors that may also affect the hypothalamic–pituitary–testicular axis include social environment, access to receptive females, handling procedures, and anesthesia (Wickings *et al.*, 1986).

In the mature rhesus, the secretion of LH is pulsatile (Adams *et al.*, 1988; Plant, 1980; Steiner *et al.*, 1980; Wickings *et al.*, 1986) in response to episodic secretion of GnRH from the hypothalamus (Carmel *et al.*, 1976). It has been more difficult to quantitate the response of FSH to GnRH since techniques used for analysis have been highly insensitive (Toivola *et al.*, 1978). Testosterone is capable of stimulating spermatogenesis in rhesus, long-tailed, and bonnet macaques, but the stimulation does not appear to be sufficient to produce normal spermatogenesis, suggesting the necessity of FSH (Wickings *et al.*, 1986). Arslan *et al.* (1986) measured changes in circulating levels of FSH, LH, and testosterone in rhesus males and the responsiveness of these hormones to GnRH challenge. FSH was markedly higher in adults when compared to juvenile and pubertal males (16.1 $\pm$ 1.8 ng/ml versus 2.4 $\pm$ 0.8 and 6.4 $\pm$ 1.8 ng/ml, respectively). It was also noted that the GnRH challenge induced a significant rise in LH and testosterone but did not appear to elicit a response in FSH in all three groups evaluated. In contrast, FSH levels were increased by ~20% in the chimpanzee following GnRH administration (Hobson and Fuller, 1977).

The role of FSH has also been studied in three macaque species (rhesus, bonnet, and long-tailed) by immunization with heterologous FSH preparations. Rhesus males immunized with ovine FSH showed reduced sperm counts post-treatment, and testicular biopsies 1 year later revealed a decrease in seminiferous tubule size and, in some cases, the presence of Sertoli cells only (Wickings and Nieschlag, 1980c). Additional studies with passive immunization of this species with an homologous anti-ovine FSH preparation resulted in the reduction of germ cell numbers (Wickings and Nieschlag, 1980b). In both of these studies, serum testosterone levels were not different from controls. Similar results were observed in the bonnet monkey passively immunized against FSH and in the long-tailed macaque actively immunized with either ovine FSH or its β-subunit (Madhaw Raj *et al.*, 1982; Moudgal, 1981). It has been shown that a twofold increase in the number of germinal cells in the seminiferous epithelium occurs in long-tailed macaques in response to a twice-daily treatment of 15 IU FSH for 28 days (van Alphen *et al.*, 1988); similar findings were observed in the rhesus after 16 days of treatment. Highly significant increases were noted in the numbers of spermatogonia, spermatocytes, and spermatids. These results suggest that, in primates, FSH levels determine the number of germ cells in the testis.

Steroid hormones, particularly testosterone, may account for the red color of the sex skin noted in some nonhuman primates. High doses of testosterone will restore pigmentation in nonbreeding males to the appearance of a normal, in season, adult (Vandenbergh, 1965). In contrast, the sex skin of the talapoin, which is blue, is unaffected by castration, even when hormone levels decline to <1 ng/ml (Dixson and Herbert, 1974). The blue coloration has been attributed to melanin-like pigment in the dermis and, therefore, appears to be unrelated to testosterone. It would appear that sex skin may only be hormonally regulated in seasonally breeding monkeys (Wickings *et al.*, 1986).

4. Seasonality

Several macaques are seasonal breeders (*M. fuscata, M. mulatta, M. sylvana*), whereas many species will breed all year round (*M. arctoides, M. fascicularis, M. nemestrina, M. radiata, P. cynocephalus*, the great apes). For seasonal breeders such as the rhesus, spermatogenesis occurs during the autumn and winter months, and includes growth of the testes. During the seasonal months, a threefold increase in tubular diameter (Conaway and Sade, 1965) and increases in sperm production occur (Zamboni *et al.*, 1974), whereas during the nonseasonal months the seminiferous epithelium regresses significantly (Conaway and Sade, 1965). Wickings and Nieschlag (1980a) noted that during the rhesus nonbreeding season, testicular volume decreased to half its size and that the seminiferous tubules were depleted of spermatocytes and spermatids. Measurements of pituitary function indicate few LH pulses and little diurnal rhythms (Wickings *et al.*, 1986). During recrudescence, basal LH concentrations increase with maximal levels achieved prior to the in-season period. The number of LH pulses per 24 hr decreases to roughly four to six per day in season, with pulses occurring more frequently during the dark phase of the 24-hr period (Plant, 1980; Wickings *et al.*, 1986). Serum testosterone parallels LH throughout the year with maximum levels observed during the in-season phase only. Although the Leydig cells retain their capacity to respond to trophic stimuli throughout the year, full secretory capacity will only be noted during this time.

As observed with females, reproductive seasonality is related to particular environmental cues, although the specific mechanisms involved remain to be elucidated. Photoperiod and temperature variation do not appear to be important factors since laboratory-housed animals in controlled temperature and lighting environments will still remain seasonal. Wickings *et al.* (1981) suggested that seasonality may be mediated through the

hypothalamus since stimulation of the testes and ejaculatory behavior will occur when out-of-season macaques are administered GnRH.

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CHAPTER 10

Housing

Stephen T. Kelley and Arthur S. Hall

I. HISTORY

When medical laboratories began utilizing nonhuman primates in the early 1950s, caging techniques became an important factor in the life of captive nonhuman primates.

Early caging techniques used by trappers in India and South America led to fighting and disease transmission. Trappers would hold groups of primates in large enclosures (gang cages) until the primates could be shipped to their next destination. The primates were transported from the forests in groups of 10 to 20 animals. These small groups were often combined into larger groups of 50 or more primates after they arrived at an exporter's facility. Animals were not segregated by sex, size, age, or family groups; therefore, animals trapped from diverse geographical areas were caged together in the exporter's facility. Common medical problems such as diarrhea, tuberculosis, pneumonia, and malnutrition were observed as a result of these trapping techniques and overcrowded caging conditions. Exposures to human populations and human diseases added to the medical problems of the animals. The diet offered to the newly captive primates was often very different from their natural diet.

NONHUMAN PRIMATES IN BIOMEDICAL RESEARCH: BIOLOGY AND MANAGEMENT

Diarrhea and malnutrition were seen as a result of the dietary change after captivity.

Prolonged transportation periods from the trapping area to the export facility were common. This resulted in the primates being held in these crowded conditions for extended periods of time before they arrived at the research laboratory. On arrival at the quarantine facilities of the importer, shipments of animals from different countries would occasionally be mixed together. This mixing of animals facilitated disease transmission. With the increased use of nonhuman primates in biomedical research, exporters and importers began designing and constructing quarantine facilities to limit the transmission of communicable diseases.

The original cages used for housing monkeys individually were modified chicken or turkey cages constructed of galvanized wire. Originally, groups of monkeys were housed in modified dog runs. When Congress established the Regional Primate Research Centers Program in 1963, a national nonhuman primate health program began to emerge. As veterinarians became more involved in primate care and research in the mid-1960s, new primate cage designs were developed. Cages that were too small and did not provide for the needs of the animals became the focus of discussions at primate veterinary meetings. Standards for cage sizes began to evolve.

In 1968, the National Academy of Science, Institute of Laboratory Animal Resources, appointed a subcommittee to develop standards and guidelines for the management of nonhuman primates. These guidelines were revised in 1980 [Institute of Laboratory Animal Resources (ILAR), 1980] to include three categories of primate cages. The search for the ideal caging system for primates which began in the late 1960s continues today.

Traditionally, three groups of primates have been commonly used in biomedical research: New World monkeys, Old World monkeys and the great apes.

New World primate genera such as *Saimiri, Saguinus, Tamarinus, Aotus,* and *Cebus* are small animals that live in pairs or groups. These animal benefit from the presence of perches and some require nest boxes. Many species have long prehensile tails; therefore, the original cages were designed to provide considerable vertical space.

Old World nonhuman primate genera such as *Macaca, Cercocebus, Cercopithecus,* and *Papio* range in size from a 3 kg *Macaca fascicularis* to a 25 kg *Papio anubis.* They require different types of cages, which are generally three to four times the size of the monkey that they are designed to house. These cages usually have a movable back wall to enable physically pulling the primate forward for observation, capture, or injection of anesthetics or medication.

The great apes represented by *Pan troglodytes* are housed in cages that are much larger, heavier, and more complex than smaller primate cages (ILAR, 1980).

In the early 1960s, various sizes and designs of cages to house the *Macaca* species were tested. The stainless-steel hanging cage with an underlying waste collection pan was the major result of this early testing program.

Most primate research facilities developed prototype housing configurations for various types of primates. The California and Oregon Primate Centers developed and improved outdoor enclosures such as field cages (University of California, 1979), corrals (Alexander *et al.*, 1969), and corn cribs modified for macaque breeding. The Caribbean Primate Center originally developed the island and the corral breeding programs for macaques (Kessler and Bérard, 1989). The Yerkes Primate Center developed a great ape caging program which has been further advanced by the University of Texas at Bastrop, Texas (Riddle *et al.*, 1982). Baboon caging and systems for breeding baboons were developed and tested at the Southwest Foundation for Biomedical Research (Eichberg *et al.*, 1979).

Various pharmaceutical companies, contract research laboratories, universities, primate centers, cage manufacturers, importers, and exporters have all contributed to the development of the current primate caging systems. In addition, exporters and importers improved the cages used for shipping animals.

Federal regulations and guidelines have established minimum standards for housing nonhuman primates (Animal Welfare Act, 1985; National Institutes of Health, 1985). The 1985 amendments to the Animal Welfare Act included a requirement for institutions to develop, document, and follow an appropriate plan for environmental enhancement to promote the psychological well-being of nonhuman primates. This included the requirement to address social grouping and environmental enrichment. Special considerations must be given to infants, juveniles, and animals exhibiting signs of psychological distress. Primates with experimentally imposed restrictions on activity or housing, great apes weighing over 50 kg, or animals being maintained in restraint devices must be given special attention. These regulatory requirements have resulted in a variety of novel housing strategies, cage designs, and housing systems (Bayne, 1989; Bielitzki *et al.*, 1990).

II. PRIMARY HOUSING DESIGN

The following factors should be considered in the design of primary housing: (1) the cage should be designed to promote physical comfort of the animals being maintained, (2) the cage should be compatible with normal growth and development and the prevention of disease, (3) the cage should be designed to facilitate sanitation and proper maintenance, (4) the cage should meet the research and primate care requirements, and (5) the cage should be designed to meet the requirements of regulatory agencies.

A. Common Types

The types of primary enclosures selected for nonhuman primates depend on numerous factors, including animal species, animal age, the types of research programs, personnel safety, animal safety, experience of the staff responsible for animal

I

TERMINOLOGY USED TO IDENTIFY CAGES

Basis	Common name
Cage type	
Single male/single female	Individual or pair housed caging
Single male/multiple female	Harem caging
Multiple male/multiple female	Group, gang, or troop caging
Cage location	
Indoors	Single/paired/runs/gang/harem
Outdoors	Corral/field cage/corn crib/island
Indoor/outdoor	Runs

care, economic considerations, regulatory requirements, degree of urbanization surrounding the facility, and climate of the facility location. Table I lists the common terminology used to identify cages.

Nonhuman primates are a very diverse group of animals. Over 230 species and subspecies of nonhuman primates ranging in size from approximately 120 gm to 165 kg are recognized by taxonomists (Napier and Napier, 1985). Some species, such as the gibbon, are almost totally arboreal, whereas others, such as the macaques, are both tree dwelling and terrestrial. Some primates, like baboons, are mostly terrestrial. Owl monkeys and many of the prosimians are nocturnal whereas other primates are diurnal. Many nonhuman primates such as the macaques live in troops. Other species, such as the marmosets, live in breeding pairs. Some species can only survive in very limited tropical habitats whereas others tolerate a wider range of temperatures. Some primates may adapt to severe winter conditions. These species differences have a major impact on the type of housing required to adequately care for the animals.

The type of program which utilizes the animals has an impact on the type of housing selected for primates. Animals in production colonies may be housed in large enclosures capable of holding multiple animals and their progeny. Some research protocols may require that animals be housed individually. Other protocols may require that animals be housed in cages to allow close observation and access; however, depending on the experimental design, these animals may be housed in pairs or small social groups throughout the experiment.

The selection of the animal housing method is dictated by the research requirements and financial resources. The location of an institution and climate of the area affect the type of housing which may be employed. For example, an institution located in a densely populated urban area may not have the option of housing animals in outside enclosures. Local, state, or federal regulations regarding environmental impact may affect the type of facilities which can be used to house nonhuman primates. Nonhuman primate housing methods may be dictated by the experience of the institution with certain types of cages.

II

ANIMAL WELFARE ACT NONHUMAN PRIMATE CAGE SIZE STANDARDS

Group	Weight		Floor area/animal		Height	
	lb	kg	Square feet	m^2	inches	cm
1	<2.2	<1	1.6	0.15	20	50.8
2	02.2–06.6	1–3	3.0	0.28	30	76.2
3	06.6–22.0	3–10	4.3	0.40	30	76.2
4	22.0–33.0	10–15	6.0	0.56	32	81.28
5	33.0–55.0	15–25	8.0	0.74	36	91.44
6	>55.0	>25	25.1	2.33	84	213.36

B. Cages

Nonhuman primates may be housed in cages to allow close access for health observations or to fulfill investigative or management requirements. Animals may be housed in cages individually, in pairs, or larger groups. If frequent handling of animals is required, it may be necessary to house nonhuman primates in cages with movable restraint backs so they can be safely restrained for injections or other procedures. Cages with restraint devices may be necessary to limit potential personnel exposure to some of the zoonotic diseases such as *Herpesvirus simiae.*

Cages available from commercial sources are sometimes custom designed and constructed according to specifications supplied by the purchaser. Existing designs may be modified to suit special primate species requirements, research program needs, or institutional requirements. Some institutions have the capability to fabricate or modify cages for specialized requirements.

C. Size

The federal government has established minimum standards for cage sizes for nonhuman primates (Table II). These standards were initially published as recommended guidelines by the National Institutes of Health in 1985 and adopted as regulatory standards by the U.S. Department of Agriculture (Animal Welfare Act, 1985). These standards should be considered as minimum requirements. Discretion and professional judgment should be employed in the selection of cage sizes. Cages should allow for the normal conformation and growth of the animals.

Low perches that do not allow animals access to the floor are considered part of the floor space. If two or more animals are housed in the same enclosure, the cage floor area must be the sum of the space required for both animals and the cage height must meet the minimum height requirement for the largest animal. An exception to this is that mothers with infants less than 6 months of age may be housed together in primary enclosures that comply with the minimum floor area and height standards for the mother. The 1985 amendments to the Animal Welfare Act allow exceptions for innovative enclosures that do not comply with the minimum space requirements; however, these

innovations must allow sufficient space and opportunity for the animals to express species typical behaviors. These exceptions and modifications must be approved by the institutional animal care and use committee or by the U.S. Department of Agriculture.

D. Design and Construction

The U.S. Department of Agriculture has established facilities construction and operating standards (Animal Welfare Act, 1985) for nonhuman primate housing. The Animal Welfare Act requires that primary enclosures, whether they are individual cages or large enclosures designed to house groups of animals, must comply with the minimal requirements summarized as: (1) must be designed and constructed to be structurally sound for the species of nonhuman primates contained in them; (2) must be kept in good repair; (3) must have no sharp surfaces that could injure animals; (4) must protect animals from injury; (5) must contain animals securely and prevent accidental opening by animals or personnel; (6) must keep other unwanted animals from entering the enclosure or having physical contact with nonhuman primates; (7) must enable animals to remain clean and dry; (8) must provide shelter and protection from extreme weather conditions; (9) must provide shade to shelter all animals simultaneously in the enslosure; (10) must provide animals with easy and convenient access to clean food and water; (11) must provide that all surfaces which may come in contact with the primates be easily cleaned, sanitized, or replaced when worn or soiled; (12) must have floors constructed in a manner that protects nonhuman primates from injuring themselves; and (13) must provide sufficient space for nonhuman primates to make normal postural adjustments with freedom of movement.

E. Construction Materials

Nonhuman primate cages should be constructed of materials that are nontoxic. They must be resistant to rust, corrosion, and withstand routine sanitation and maintenance procedures. Cages must be adequately constructed to contain the animals safely. Cages are usually constructed of stainless steel or, less frequently, aluminum. Galvanized metal which was used in the past to manufacture individual cages is seldom used now. Newly developed plastic materials may provide useful alternatives for cage construction (Kessler, *et al.*, 1994).

Galvanized metal is the least expensive material for cage construction but has a shorter life expectancy than aluminum or stainless steel. The zinc coating oxidizes when exposed to frequent washing at the minimum sanitization temperature of 180°F. The underlying metal rusts and disintegrates at these temperatures. If exposed to acid agents, the zinc coating will deteriorate even more rapidly and expose the underlying metal. The hot dipped galvanizing process may leave sharp points which must be blunted by grinding or sanding. The blunting process may expose the underlying metal. The zinc component in galvanized cages may prove toxic to some nonhuman primate species (Obeck, 1978; Stevens *et al.*, 1978). This exposure to zinc has been reported to be a cause of severe anemia in nursing infants. The clinical syndrome associated with the zinc toxicity in infants was commonly referred to as the "blonde baby" syndrome because the hair of the affected infants was usually blonde in color. It has been observed that nursing mothers and teething infants lick the zinc-coated cage surfaces and consume small amounts of zinc. The zinc is excreted in the mother's milk, causing zinc toxicity in nursing infants.

Aluminum is comparatively lightweight and is easily fabricated into cages, but lacks the strength of galvanized metal or stainless steel and is more easily damaged. Routine moving, sanitization procedures, and animals chewing on the cage may damage aluminum cages. Aluminum requires more material to be used in cage construction because it lacks structural strength. This extra material restricts light from entering the cage and limits visualization of animals by personnel. Aluminum oxidizes when exposed to routine sanitization procedures. This does not affect the utility of equipment constructed of aluminum but it may become stained and unsightly. Cages constructed of aluminum may have an expected life of 5 to 10 years depending on the alkalinity of detergent and disinfectants used to sanitize the equipment. Aluminum equipment will last longer if sanitized with chemical agents at pH 11 or greater.

Stainless steel is the most durable material for nonhuman primate caging. This material has an indefinite life expectancy. In order to provide adequate strength and resistance to rust and corrosion, cages should be manufactured entirely of Type 304 stainless steel. All welds should be smooth and electronically polished.

F. Design Criteria

1. Species Considerations

Most cage design considerations listed in this chapter are for the more commonly used macaque species. Guidelines for caging Old World and New World species are similar; however, New World nonhuman primates may require more cage height because these animals usually have long tails and are adapted to an arboreal lifestyle. Perches and climbing apparatuses should be provided in New World primate cages. These cage accessories increase the functional space in the cage. Marmosets, tamarins, and owl monkeys should be provided with nesting boxes.

Cage sizes for large species must also comply with the federal regulations. For example, small baboons may be housed in cages designed for the group 3 or group 4 macaques. Large baboons or chimpanzees may have to be housed in much larger and stronger enclosures which are specifically designed for these species.

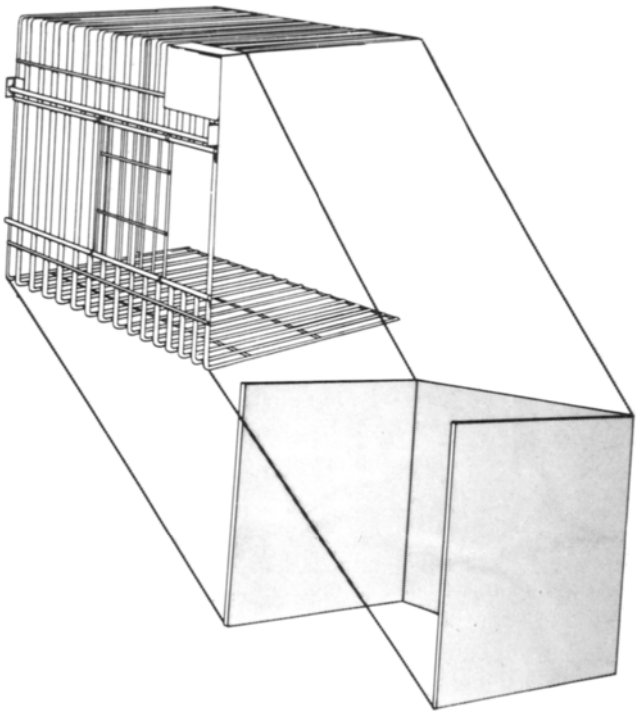

Fig. 1. Common cage construction technique with two U-shaped units welded together to form a box-type cage.

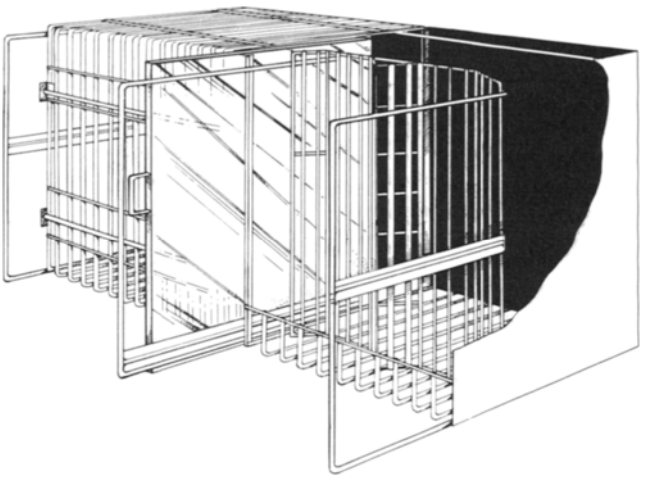

Fig. 2. Cage showing a movable back wall "squeeze back" and a removable center partition.

Cage design and construction may vary for different species. Cages designed for housing macaques may be constructed of 8-gauge stainless-steel wire welded on a 1-inch × 3-inch grid pattern for the front and top. The bottom pattern is commonly a 1-inch × 1-inch grid of 8-gauge welded wire. This floor grid pattern is designed to retain food and prevent injuries.

The most commonly used individual nonhuman primate cages are designed with a solid sheet of stainless steel bent into a U shape to form the sides and back. These solid sides were originally designed to prevent injuries from animal-to-animal contact in adjacent cages. A grid of welded wire is also bent into a U shape in order to form the bottom, front, and top of the cage. The two U-shaped units are welded together to form a cage (Fig. 1). A movable cage wall may be added to the back of the cage to assist with safe restraint and handling (Fig. 2). Cages may be further modified with a removable side partition or door to allow the animal access to a larger cage area or to house two or more animals together (Fig. 2). Cages have also been designed to allow groups of four rack-mounted cages to be joined together to form one large cage.

Cage doors may be designed like a guillotine, which slide up and down, or which slide from side to side. Swinging hinged doors are not recommended because the animal is more likely to escape when the animal is being transferred or captured. Selection of cage door design is largely dependent on the facility and equipment limitations. Personnel experience and preference are also important factors to consider when designing a cage door. Cage door locks should be strong and "tamper-proof." It may be necessary to install padlocks or other securing devices on the cage doors of animals that become particularly clever and escape frequently.

2. Cage Support System

Cages may be wall mounted with brackets. They may also be supported by movable cage racks (Fig. 3). Wall-mounted systems require the cages to be washed in place or removed from the bracket system. They may be removed by lifting them from the supporting brackets, either manually or with the aid of a mechanical hoist. Wall-mounted cage systems may be less costly than the cage rack system; however, the support brackets impede sanitation and provide harborage for vermin. As cages become larger and heavier, the weight and torque placed on the wall and the supports may cause damage to these structures.

Rack-mounted cage support systems may be designed to hold from two to six cages depending on the cage size. Racks are usually mounted on casters to allow mobility and may be sanitized in a rack washer. Cage racks should be appropriately sized to move through animal rooms, corridors, and service room doors of the facility. Cage racks used to house medium to large size nonhuman primates should be designed to allow them to be secured to a wall or other permanent building structure so that animals cannot move or tip over the racks.

3. Waste Collection

Most nonhuman primate cages with welded wire floors have waste pans located under the cage floor. This may be modified in some facilities to allow the waste to fall onto the floor or into a trench where it is flushed into a drain. Waste pans should be

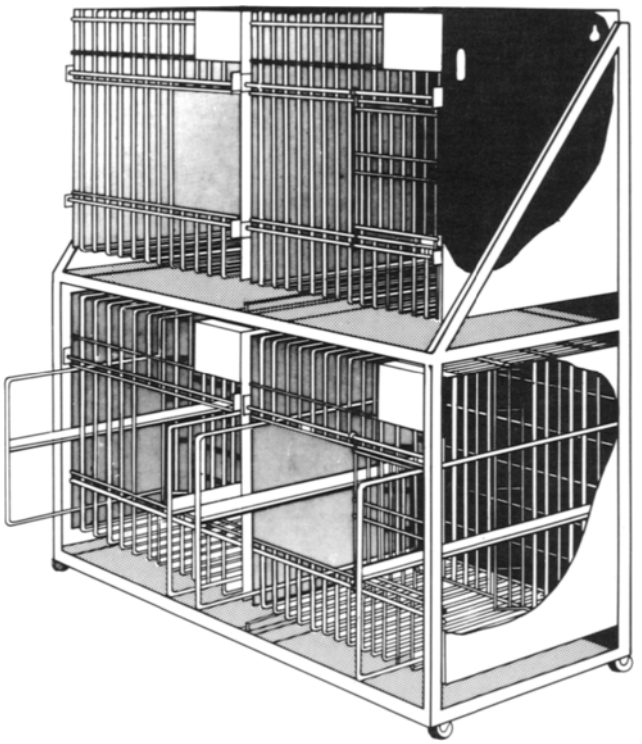

Fig. 3. Example of cages that are mounted on a movable rack.

located far enough below the cage floor so the animal cannot reach the waste pans. This may be difficult to accomplish because of the height of the entry door or the height of the room ceiling. If cages are stacked in tiers of two units, the distance available between the upper and lower cage may be limited.

a. REMOVABLE PANS. Waste may collect in individual removable pans located underneath the cages. Pans are then removed for dumping waste and sanitization. Waste pans should not be cleaned in animal rooms. Absorbent noncontact bedding may be used in the pans to absorb moisture.

Advantages of removable pans are the reduction of the potential for transfer of disease between cages and the minimization of creating aerosols during sanitization procedures. Sanitization is best accomplished in a mechanical washer.

Disadvantages of removable pans are that they may be difficult to transport, are more labor intensive, and may require extra equipment for frequent changing.

b. FLUSH PANS. Flush pans can be added to wall-mounted or rack-mounted cage systems. Flush pans must be sloped so that water flow is directed toward a drain. Pans should be sloped front to back rather than side to side. A side-to-side sloped pan allows waste from one cage to flow underneath other cages before reaching the drain. This may allow an animal to reach down into the pan and contact the waste from other animals and increase the risk of disease transmission. A front-to-back slope reduces this potential because waste flows a short distance to the back of the pan and into a gutter, thus is inaccessible to animals. Water flush pans are less labor intensive to clean than individual removable pans. Waste pans can be flushed frequently to reduce odors.

Automatic water flush systems for waste pans are not commercially available and must be locally designed and constructed. A flush system requires large volumes of water being released at one time to provide adequate flushing action.

4. Watering

Water may be provided to animals in individual cages in bottles, bowls, or with an automatic watering system. Water bowls are not recommended because of the potential for spillage and contamination. Water bottles may be economical to use for small numbers of animals but are not practical when larger numbers of animals are maintained. Water bottles are labor intensive and require frequent sanitization, reassembly, and refilling. When water bottles are used, it is easy to determine if animals are drinking. Bottles provide a route for the administration of medications, dietary supplements, and test substances.

Automatic watering systems deliver water to an "on demand" stem valve watering device usually located at the back of the cage. The water device should be located so that it can be easily reached by the animals even if they are unable to stand or climb. Automatic water systems must be designed to prevent back flow and to allow periodic system flushing and chemical treatment of the watering system. The primary advantage of automatic water systems is the cost savings from reduced labor. Disadvantages are the potential system failures and maintenance problems, inability to easily determine if animals are drinking, and the potential for disease transmission from animal to animal through the water lines. These disadvantages can be largely overcome through proper system design, installation, maintenance, and monitoring.

5. Feeding

Food may be provided in food cups or bowls attached to the cage. Frequently, nonhuman primates will remove all the food from the food container and drop it on the floor. Some commercial diets are manufactured into large biscuit shapes that prevent them from falling through the wire floor. Various strategies have been developed to provide food in ways to improve the psychological well-being of the animals. Many of these strategies use devices that allow animals to engage in foraging type behaviors (Bayne *et al.*, 1991; Bloom and Cook, 1989).

6. Cage Accessories

a. FALSE BACKS. Movable false back panels (often referred to as "squeeze backs") are recommended as a method to restrain nonhuman primates easily, gently, and safely against the

front of the cage. Movable back panels may not be desirable for cages that house small primates because the animals may become trapped between the false movable panel and the back of the cage.

b. PERCHES AND ENRICHMENT DEVICES. Perches are recommended for most nonhuman primate cages. Federal regulations suggest that perches provide additional cage enrichment. Perches should be easily sanitized and constructed of stainless steel, aluminum, or polyvinyl chloride (PVC) pipes. A large variety of other environmental enrichment devices or toys may be mounted or placed in the cage to allow animals to engage in manipulative and play behaviors. Examples of these devices include malleable rubber or plastic toys of various shapes, swings, and aluminum rings. The range of devices that may be purchased or easily constructed are limited only by the resources of the institution and the imagination of the animal care staff.

c. TRANSFER CAGES. Transfer cages should be designed to allow easy movement of animals from their home cage into the transfer cage.

III. GROUP HOUSING

Group housing of nonhuman primates usually implies more than two animals in a primary enclosure. Group-housed nonhuman primates may be located in three types of facilities: indoor facilities which are totally environmentally controlled, indoor/outdoor facilities in which only the indoor portion is environmentally controlled, and outdoor facilities which are not environmentally controlled. Major factors in the selection of the housing method for groups of nonhuman primates are the climate encountered at the institution and the species being housed. The local climate is of paramount importance in determining if outdoor enclosures are feasible. Since the provision of shelter from environmental elements is a regulatory requirement, adequate shelter must be provided. The shelter(s) must be designed so that all animals can seek shelter simultaneously. Animals in outdoor group enclosures should have opportunities to seek an adequate environment to assist in controlling body temperature. For example, animals may modify their immediate surroundings by huddling in sheltered areas with other animals, thus reducing the loss of body heat. They should be allowed to seek the sun or shade, climb above the ground, or use the water sprinklers in hot weather to keep cool. If supplemental heat is provided, the heated area must be adequately ventilated with fresh, nonrecirculated air because the animals tend to overcrowd into these areas. Heated areas that are poorly ventilated may result in wet floors, walls, and increased incidence of disease. Indoor group housing facilities are comparatively costly to build and maintain. Indoor/outdoor facilities may be somewhat less costly to construct and operate than indoor facilities; however, all animals must have access to the environmentally controlled indoor portions of the facilities. Outdoor facilities provide the most economical alternatives for housing large numbers of animals in group sizes and configurations which may approximate those found in the natural state.

A. Criteria for Selection of Facilities

Factors to consider when selecting indoor, indoor/outdoor, or outdoor facilities for group housing nonhuman primates include species, local climate, ventilation, environmental enrichment, construction, food and water provision, pest control, animal observation, animal handling, and security.

Consideration of the nonhuman primate species to be group housed may determine if outdoor housing is feasible. Certain nonhuman primate species are less tolerant of cold weather than others and cannot be easily housed outside in some geographical areas without provisions for supplemental heat. The primate species and their behavior patterns affect the size and strength of the enclosure. For example, brachiating species require a large amount of vertical height and space to swing. Arboreal species require more vertical space, whereas more terrestrial species require a combination of climbing area for play and ground area for foraging. Adult terrestrial species may require less climbing structures, but young animals may benefit from these environmental enhancement devices. The age of the animals may determine if outdoor facilities are feasible during certain times of the year. For example, infants and aged adults have more difficulty adapting to sudden climate changes.

Federal regulations (Animal Welfare Act, 1985) require that large enclosures designed to house groups of animals must comply with minimal housing requirements. A shelter is defined as a facility which provides the animals protection from inclement weather and temperature extremes at all times. The sheltered portion of the enclosure must provide adequate light, must have heat available, and must provide adequate ventilation when required to protect primates from cold temperatures. The sheltered facility may be totally enclosed or the shelter may be an indoor facility connected to an outdoor enclosure. An outdoor housing facility is defined as any structure, building, land, or premise intended to house animals in which temperatures cannot be controlled within set limits. Both sheltered and outdoor housing must provide adequate protection from inclement weather. The shelter must be of sufficient size to comfortably hold all nonhuman primates in the facility at one time. There must be multiple shelters or other means to ensure protection of subordinate animals from aggressive or dominant animals.

B. Enrichment

Group housing enclosures should include environmental enrichment devices. Various climbing devices or jungle gyms provide animals with exercise and play opportunities to engage in

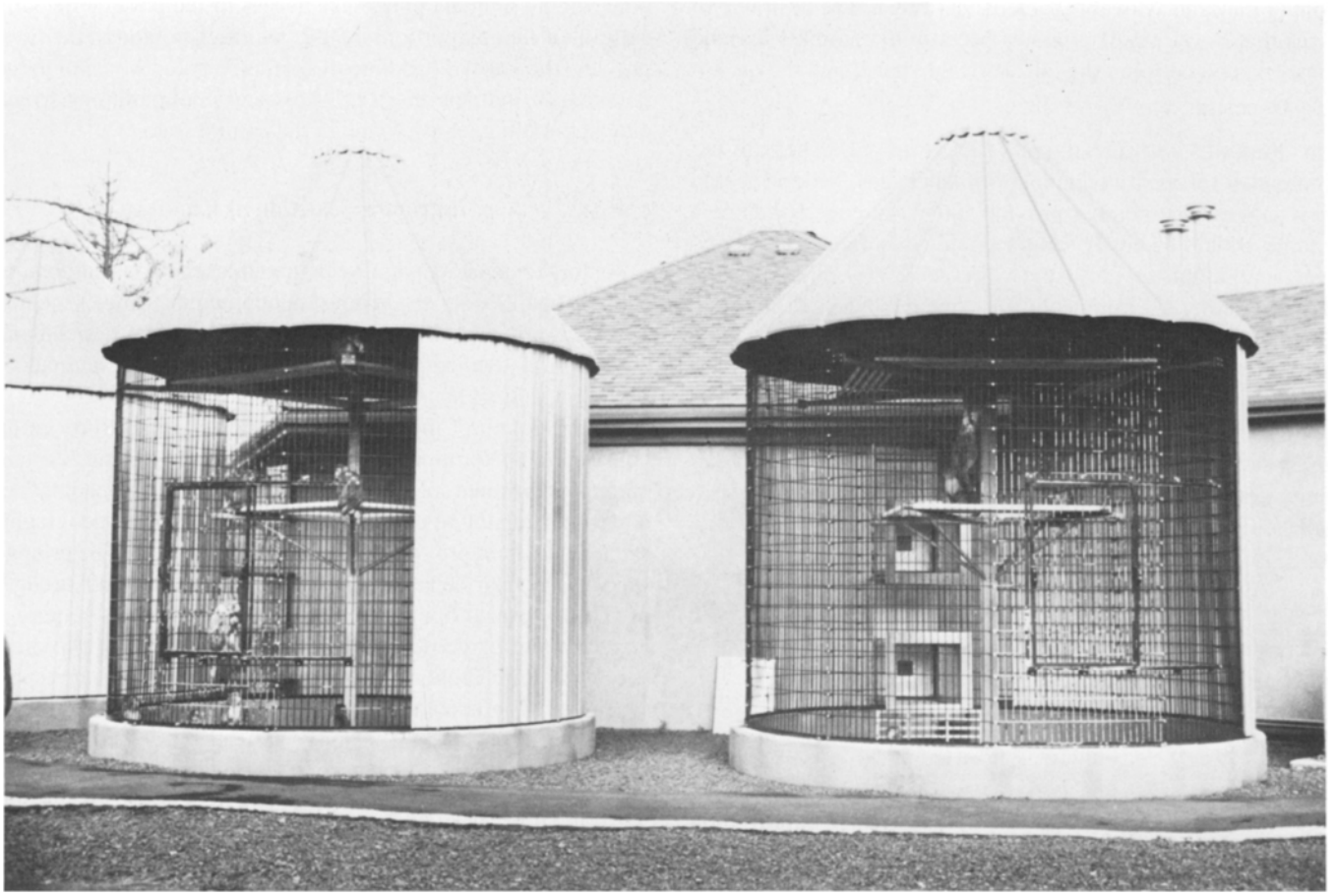

Fig. 4. Example of an outdoor circular corn crib enclosure.

species typical behaviors while increasing the "effective use area" of the enclosure. Climbing devices may pose a risk of trauma when animals jump or fall from high structures. Resting surfaces which allow animals to sit above the ground surface in the sun or shade should be provided. Other environmental enrichment devices include hiding places and sight barriers to reduce incidents of aggression and trauma. Play devices such as sticks, balls, and plastic barrels also may help prevent fighting and aggression. Environmental enrichment devices may be natural or artificial. Devices made from natural materials such as wooden logs or trees are realistic and economical; however, they are difficult to clean and may deteriorate rapidly. Natural products must be removed and replaced periodically. Artificial devices may last longer, are usually repairable, are sanitizable, and may allow for innovative design. Artificial devices may cost more than natural devices. Animals in group housing enclosures should be provided foraging opportunities when possible which allows them to engage in species typical behaviors. Foraging opportunities can be provided by using natural vegetation and by scattering foraging materials such as feed grains on the ground. Feed grains can be placed on the enclosure floor in deep beeding material such as wood chips, sawdust, shredded paper, or straw. The use of feeding puzzles and other food rewarding devices placed strategically around the group enclosure may also provide environment enrichment (Bloom and Cook, 1989).

C. Types of Enclosures

Commonly used outdoor enclosures are corn cribs, runs, islands, corrals, and field cages.

1. Corn Cribs

Small outdoor enclosures such as circular galvanized wire "corn crib" structures (Fig. 4) have proven effective for housing primates. Outdoor corn crib enclosures are usually circular, hexagonal, or cuboidal structures constructed with welded galvanized or aluminum rod walls with approximately 130 square feet of floor area and covered with a sheet metal roof. Corn crib outdoor enclosures may be placed on concrete or cement block

footings with cement or gravel floors. They can also be constructed with elevated metal or plastic grid floors which reduce animal contact with waste material and moisture. Corn cribs may be designed to be movable.

2. Runs

Indoor/outdoor-type enclosures referred to as runs (Fig. 5) have often been utilized to house primates. Runs can be of various shapes and sizes. The inside portion should be of sufficient size to house adequately all of the animals simultaneously. Runs should be large enough to prevent dominant or aggressive animals from keeping others from food, water, and access to shelter. Like corn cribs, runs may have cement or elevated grid floors.

3. Islands

Islands serve as outdoor facilities in locations with warm climates. The water barrier limits the number of animals that escape from the island, however, macaques are good swimmers and can escape from islands that are located near other masses of land. Since chimpanzees do not swim, islands and water barriers are dangerous because the animals may drown. Electrified fencing has been used to supplement island or terrestrial enclosures with variable success.

4. Corrals and Field Cages

Outdoor group enclosures may be configured as open-topped corrals (Fig. 6) or as totally enclosed field cages (Fig. 7). These enclosures vary in size from less than an acre to several acres in size.

Open-topped corral enclosures should be surrounded with solid galvanized panels that are 11 feet tall and tilted inward at a 15° angle. At this inward angle, the height of the slanted wall from the ground to the top is approximately 10 feet 8 inches. Corral walls that are 10 feet high without the inward angle must be capped at the top of the corners with a metal panel to prevent animal escape. The wall panels must be joined together to provide a smooth interior surface which cannot be climbed. The pipes supporting the panels should be set in a concrete footing. There should be a small gap (2 inch) at the bottom of the panel wall to allow airflow but prevent escape by young animals. In some warm climate locations, the bottom half of the wall can be constructed of chain link wire to allow for better circulation of air and to prevent the wall from being damaged by strong winds.

Enclosures that are sometimes referred to as field cages may be built entirely of chain-link fencing material (Fig. 5). This type of construction requires that a chain-link fence roof be constructed to prevent the escape of animals. The galvanized walls of outdoor enclosures, whether sheet metal, chain link, or welded rod, will eventually rust and require refurbishing or replacement. Rust can be delayed by preventing moisture, soil, organic debris, rocks, and gravel from prolonged contact with the galvanized surfaces of the enclosure. Although considerably more expensive, enclosures constructed of aluminum will not rust.

D. Ground Surfaces and Floors

Ground surfaces in the outdoor facilities may be covered with concrete, rock, gravel, soil, natural vegetation, or elevated wire. Federal regulations require that floors in outdoor enclosures be raked and cleaned frequently enough to prevent the accumulation of feces and organic debris. Excessive accumulation of waste material may create a health hazard to the animals.

1. Soil Surfaces

Large enclosures with soil surfaces must have adequate slope and/or drain tiles to assure drainage of excessive surface groundwater. In some areas, state regulations may require that runoff from animal housing enclosures be contained and prevented from entering designated wet land areas or water sheds. A soil surface is only practical in very large enclosures such as field cages, corrals, or islands. If possible, the soil surface should be planted with grass or other vegetation appropriate to the area. Grass and vegetation provide a medium for foraging and grazing behavior which may reduce aggressive behavior. The number of animals allowed in enclosures planted with vegetation should be appropriate to the enclosure size so that the vegetation will survive the foraging and grazing activities. Irrigation must be provided to support the vegetation during periods of drought. It may be beneficial to consult local agriculture extension agents or pasture specialists regarding the selection of vegetation, soil fertilization, and weed and pest control programs. Areas of the soil surface around the climbing structures and shelters should be cleaned manually on a regular basis. These soil surfaces allow natural biological degradation of waste. Soil surfaces cannot be sanitized and may harbor parasites or microorganisms such as *Clostridium tetani* (Rawlins and Kessler, 1982).

2. Rock Surfaces

Ground surfaces of outdoor enclosures may also be covered with rock or gravel. The rock should be sized and shaped to prevent compaction of the rock surface. Small rock fragments may be consumed by the animals and cause digestive disturbances. Rock surfaces should be cleaned by periodic raking. Frequent removal of feces and uneaten food improves the sanitation of the ground surface. The total removal of the contaminated rock and replacement with clean rock on a regular basis is ideal. An area for safe disposal of contaminated rock must be designated. Rock for ground surfaces may be economical if the

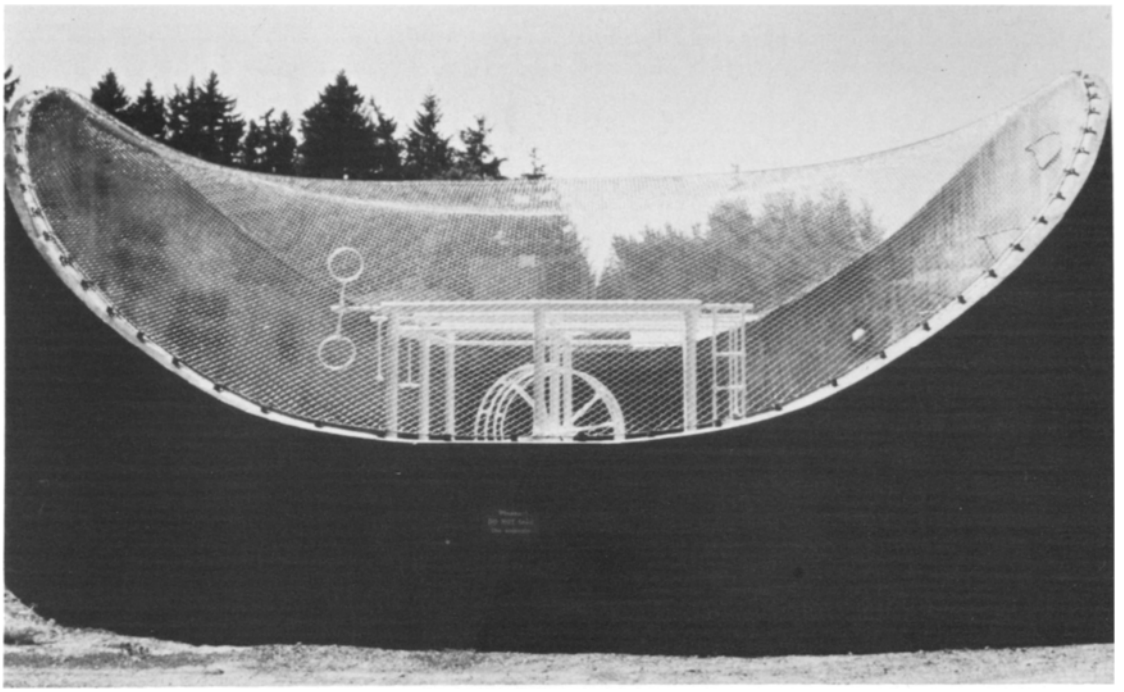

Fig. 5. Example of an indoor/outdoor run-type enclosure.

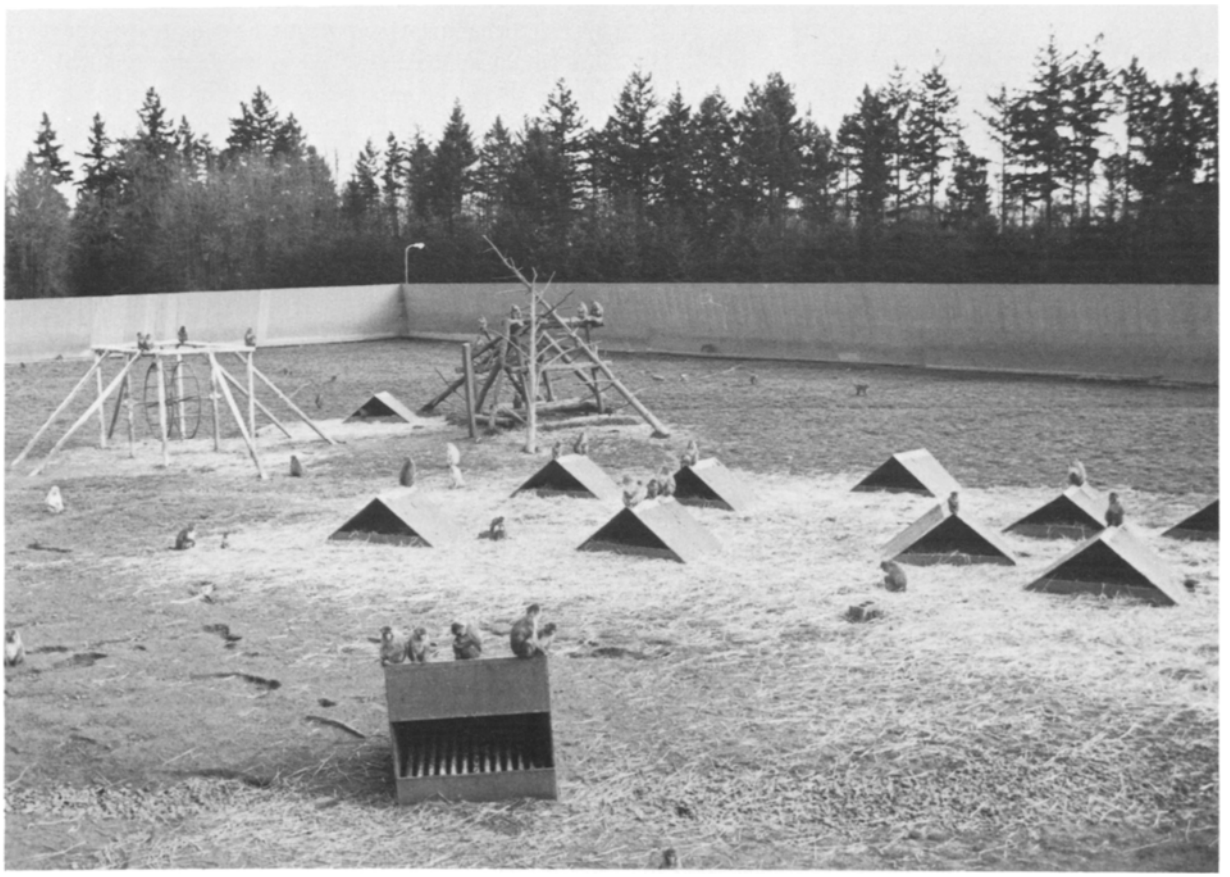

Fig. 6. Example of an outdoor corral-type enclosure.

Fig. 7. Example of an outdoor field cage enclosure.

rock is available locally. In some states, rock surfaces may require a sewage collection system.

3. Concrete Surfaces

Concrete floor surfaces are commonly used for smaller sized outdoor or indoor/outdoor group housing enclosures. The floors should be finished to provide a smooth, sealed, seamless, and water impervious surface that is easily sanitized. Care should be taken during the finishing of the floor to provide a surface that is smooth enough to be easily sanitized, but not so smooth as to create a hazard to the animal care personnel or the animals. The surface of concrete floors can be coated with nontoxic materials such as epoxy resins to prolong the life of the floor. Unsealed floors can deteriorate rapidly and require routine maintenance. Floors should not be surfaced with materials that are potentially toxic to animals (McNulty and Griffin, 1976; Altman *et al.*, 1979; Geistfeld *et al.*, 1982). Concrete floors may not be desirable in cold weather because they absorb body heat. These floors should to be heated in certain climates to promote drying and to provide a warm surface for the animals. Floors require installation of drains which must be connected to a sewage system. Floors should be adequately sloped with a recommended minimum pitch of 0.25 inch/feet (2.1 cm/m). Drains at least 6 inches (15.2 cm) in diameter are recommended (National Institutes of Health, 1985). Concrete floors limit the opportunities for foraging unless they are covered with straw or other bedding material. When bedding materials are used, they must be removed on a regular basis.

4. Elevated Floor/Perch Surfaces

Elevated grid floors may be installed in small group housing enclosures to keep the animals off the ground and to simplify the sanitization procedures. Grid floors may be constructed of metal, fiberglass, plastic, or PVC. Galvanized metal grid material should be avoided to prevent zinc toxicity (Obeck, 1978; Stevens *et al.*, 1978). During freezing weather, direct exposure of animals' skin to metal surfaces may cause dermal injuries because the skin of the hands, feet, or tongue may adhere to the metal surface. Perches and other raised surfaces may be constructed of metal pipe which is covered by a sleeve of PVC pipe. PVC is easily sanitized and is resistant to destruction. PVC-covered perches are more comfortable for primates in cold weather than metal perches. This type of surface can last for many years with minimal maintenance. When constructing perches with this material, any exposed metal should be aluminum or stainless steel to prevent rusting.

E. Animal Observation, Identification, and Handling

The observation and handling of nonhuman primates housed in large enclosures are more difficult and complicated than for

cage-housed animals. The degree of difficulty is directly proportionate to the size and complexity of the enclosure. Animals housed on islands with natural vegetation are usually observed and captured when they are fed in enclosed feeding stations. Animals housed in corrals or field cages must be observed from towers, observation points, or by walking through the enclosure. Close observation of primates in large enclosures is difficult and may require the assistance of binoculars. Animals housed in smaller enclosures such as indoor/outdoor runs or corn cribs are easier to observe because the structure restricts their ability to move away from the observer.

The capture of animals housed in group enclosures may be accomplished by moving animals through a tunnel device which allows animals to be separated and transferred into transport boxes. Capture of a primate that must be separated from a large group may be difficult and may require the use of nets and extra personnel. Capturing animals in group enclosures is more hazardous to personnel than removing animals from individual cages. This potential hazard can be reduced by (1) using trained, experienced, and well-supervised personnel, (2) using properly designed equipment and facilities, and (3) training the animals so that capture procedures are a familiar event.

Primates in group enclosures may be marked with symbols to assist with identification. Animals may be identified by symbols painted on the hair coat with a dye that will remain on the hair from 1 to 6 months. The amount of time that the dye remains on the hair depends on the season of the year and the hair shedding cycle. Face tattoos may also be used but may be difficult to read. To identify face tatoos, a clear view of an animal's face is required. A suntanned face and facial hair obscure facial tatoos. Subcutaneously implanted electronic devices are being used with mixed success. The subcutaneous implants may migrate to different anatomical locations after being implanted. After several years, the implants are sometimes difficult to locate.

Disease control and health monitoring are difficult in group-housed primates because of the limited ability to closely observe and capture these animals. These difficulties can be diminished with trained, motivated, and experienced personnel who can identify and remove sick and injured animals for veterinary care.

F. Watering

1. Continuous Flow

Water may be provided continuously from a free-flowing source or from an "on demand" automatic watering device. Disadvantages of free-flowing water systems are water waste, difficult sanitization, requirement for adequate water drainage, and potential for water contamination. Advantages of this type of water system are that it is easy to observe and assure that the animals can attain water, the water does not freeze easily when running continuously, and it provides play and cooling opportunities around the water fountain.

2. Automatic Devices

Advantages of the "on demand" automatic nipple watering system are that it is sanitary and that less water is used. Disadvantages of an "on demand" water nipple device system are that it has a potential for unrecognized failure, it may be damaged by the animals, and it may freeze unless adequate precautions are taken to provide additional insulation and heat.

Providing drinking water in receptacles such as tanks or buckets should be discouraged because of the potential for contamination.

G. Feeding

Food that is prepared in the form of biscuits and supplied to group-housed animals should be provided in a sheltered area and be protected from precipitation. Many primates select food by picking up one biscuit, examining it, dropping it, and selecting another biscuit. Feeding strategies should be developed to reduce food waste and contamination. Food hoppers should be designed to be easily sanitized. Depending on the group size and demographic composition, it may be necessary to provide food in multiple locations so that dominant animals cannot prevent other animals from obtaining food. Sufficient amounts of food should be provided to group-housed animals to assure that all animals are fed adequately. Food utilization should be closely monitored so that large amounts of food are not wasted or allowed to accumulate at the feeders. Food that is not consumed may spoil and attract birds and other pests. Excess food from previous feedings should be removed on a regular basis.

H. Vermin

In outdoor facilities and to a lesser extent in indoor/outdoor facilities, vermin and pest control is difficult. Rodent pests include rats, moles, gophers, and mice which can tunnel under perimeter foundations and live in the ground under cement foundations, concrete slabs, movable shelters, and play apparatus. Other pests include skunks, opossums, feral cats, dogs, and wild birds. These animals may transmit diseases which could be hazardous to the nonhuman primates housed in the enclosure.

Pest control begins with the facility design and construction. Perimeter foundations should be deep enough to discourage animals from tunneling underneath them. An underground perimeter barrier such as aluminum woven wire or panels helps prevent pests from burrowing into an enclosure. The use of rock aprons around concrete slabs may discourage rodents from tunneling under these structures. Federal regulations require that a perimeter fence be installed around a facility housing nonhuman primates to discourage the entry of large mammals such as dogs, skunks, and unauthorized personnel.

A regular pest control program should be instituted that is appropriate for the design and geographical location of the fa-

cility. The microbiological monitoring of wild-caught vermin should be part of the preventive medical program to assess the risk of disease transmission. Efforts to keep birds from outdoor facilities may include the use of models of predators such as owls. The use of sticky resins also discourages birds from perching near animal enclosures. These techniques may be marginally effective in keeping birds from the enclosure. Poison baits may provide some control of pest populations; however, poison baits should be located so that no risk of animal exposure to the toxic agents is possible. Rodent tunnels within animal enclosures may be treated with poisonous gas when the primates are removed from the enclosure. The agents selected may be regulated by state and federal laws. Some products may only be applied by licensed pest control applicators. Dead rodents must be removed before the primates are allowed access to the enclosure. Also, sufficient time must elapse to allow the poisons in the enclosure to dissipate or inactivate before the animals are returned. If the pest control program includes the use of pesticides or poisons, the effectiveness of these agents should be evaluated on a regular basis. These agents should be used on a routine basis to preclude significant increases in pest populations between applications of the poisons.

I. Security

The prevention of deliberate or accidental escape of animals should be considered in any housing system. Outdoor and indoor/outdoor primate housing facilities are more difficult to secure than indoor facilities. Island facilities may be the most difficult to protect from unauthorized personnel access. Outdoor enclosures such as corrals, field cages, corn cribs, or other group housing enclosures may be surrounded by additional perimeter fences for protection. These fences have limited effectiveness in "keeping out" determined intruders. The use of double door entries and locked doors into group housing enclosures will help prevent the accidental escape of animals. Entry doors into animal rooms should be equipped with windows to allow observation of the room from a secure location. Animal room doors should open inward to discourage animal escape. Facilities should be periodically checked to assure that doors and locks are secure and functioning properly. The outside perimeter walls should also be inspected for structural soundness to prevent animals from escaping. Nonhuman primates are ingenious at developing ways to escape from their primary enclosure. Devices placed in the enclosures for environmental enrichment should be evaluated to assure that they will not provide avenues for the animals to escape.

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CHAPTER 11

Nutrition

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I. INTRODUCTION

"Nutrition involves various clinical and psychological activities which transform food elements into body elements" (Maynard *et al.*, 1979). This definition best describes the science of nutrition, a chemistry-based discipline interacting with many of the physical and biological sciences. Nutritional status has a major influence on the growth, reproduction, and longevity of nonhuman primates as well as their ability to resist pathogenic and other environmental stresses. The availability of nutritionally adequate diets is essential for the well-being of nonhuman primates and to ensure data obtained from animals involved in biomedical research are not compromised by unintended nutritional deficiencies.

NONHUMAN PRIMATES IN BIOMEDICAL RESEARCH: BIOLOGY AND MANAGEMENT

The process of providing adequate nutrition for nonhuman primates involves establishing quantitative requirements for approximately 50 essential nutrients, formulating and manufacturing diets with the required nutrient concentrations, and managing numerous factors related to diet quality. Factors potentially influencing diet quality include the bioavailability of nutrients, palatability, or acceptance by animals, procedures involved in diet manufacture, transport or storage, and the concentration of biological and chemical contaminants. The objective of this chapter is to provide information regarding the numerous considerations required to provide captive nonhuman primate colonies with nutritionally adequate diets.

II. NUTRIENT REQUIREMENTS

An estimate of the quantitative nutrient requirements must be established for the various stages of the life cycle, i.e., growth, reproduction, or maintenance for specific animal species before a nutritionally adequate diet can be provided. The most reliable estimates of the nutrient requirements are based on the results of feeding trials designed to measure the performance of animals consuming a series of diets where the concentration of a specific nutrient is the only variable. Studies of this nature involving nonhuman primates have received little attention, therefore the quantitative nutrient requirements for most nonhuman primate species have not been well-defined experimentally. The estimated nutrient requirements for nonhuman primates are generally based on (1) the nutrient composition of diets resulting in acceptable nonhuman primate performance; (2) results derived from studies that had a nutritional component, but were not designed to establish nutrient requirements; (3) nutrient requirement data extrapolated from other animal species or humans; and (4) information obtained during observations of wild nonhuman primate populations while they are feeding.

Estimates of the nutrient requirements for nonhuman primates have been published by the National Research Council (NRC) (1978). Revised estimates of the nutrient requirements for these species are published periodically when there is sufficient new information in the peer-reviewed literature to support revisions in these estimates. Therefore, the latest report in the series contains the best estimate of actual requirements. The intent of these estimates is to provide guidelines for adequate nutrition and not to describe the requirements of a single animal or animal colony. The nutrient requirements of nonhuman primates are dynamic in that they are influenced by genetic and environmental factors. In addition, various stresses imposed by experimentation can cause changes in diet consumption which may require compensatory adjustments in dietary nutrient concentrations to ensure the provision of adequate amounts of nutrients. The dietary requirements of specific nonhuman primate colonies, therefore, are based not only on the published estimated requirements, but also on an evaluation of objectives for the colony. That is, factors influencing nutrient requirements should be identified and considered in selecting the nutrient composition of diets.

III. DIETARY DIVERSITY AMONG PRIMATES

A. Introduction

Primates in the wild typically consume a broad array of both plant and animal foods, as has been demonstrated by numerous field studies in Africa, Asia, and Latin America. However, the relative proportions of different types of food such as fruits, leaves, bird eggs, and invertebrate prey can differ greatly among species (Hladik, 1981). Harding (1981) reviewed field studies of the diets of 131 primate species and found that fruit was consumed by 90% of the species; soft plant foods such as buds, shoots, and flowers by 79%; mature leaves by 69%; invertebrates by 65%; seeds by 41%; and other animal foods (including eggs) by 37%. Unfortunately, it is difficult, if not impossible, to measure the quantitative proportions of different foods in the diets of wild primates because the amount of time spent feeding varies among species, sites, seasons, and individuals and because the amounts ingested per unit of feeding can differ greatly among different foods (Hladik, 1977; Milton, 1984; Oftedal, 1991). Hence it is usually impossible to determine what constitutes a "normal" intake of fiber, protein, calcium, vitamin A, or other nutrients in the wild.

B. Species

Despite these difficulties, there is no doubt that some primate species consume diets that are quite specialized, and these species may not thrive in captivity if their special dietary requirements are not recognized. For example, all species of the subfamily Colobinae are highly folivorous. This group includes colobus monkeys (genus *Colobus*), langurs (genus *Presbytis*), and proboscis monkeys (genus *Pygathrix*). Colobines have an enlarged and compartmentalized foregut that serves as a fermentation chamber in a manner analogous to a ruminant (Bauchop and Martucci, 1969; Chivers and Hladik, 1980; Martin *et al.*, 1985). The introduction of large amounts of soluble carbohydrates such as sugars or heat-processed starch may produce an explosive gas-producing fermentation, leading to bloat which can be fatal. Thus it is inappropriate to feed such animals solely on low-fiber extruded monkey biscuits, as is commonly done with other primate species. However, high-fiber biscuits have been formulated and tested which appear to support normal digestive function for these species, including firm stool (Oftedal *et al.*, 1982, Watkins *et al.*, 1985; M. S. Edwards, personal communication). Zoos often provide leaves of trees to folivorous primates as a source of fiber, but this can lead to impaction if inappropriate species of trees are used (Ensley *et al.*, 1982; Ullrey, 1986).

Among New World monkeys, howler monkeys (genus *Alouatta*) are relatively folivorous, but in these species there is no specialization of the foregut as a fermentation system. However, the colon and cecum are well-developed, and the latter is sacculated, allowing retention and fermentation of fibrous material (Milton and McBee, 1983; Crissey *et al.*, 1991). These species may also benefit from higher fiber levels in their feed.

Many small primates consume substantial numbers of insects and other invertebrates in the wild (Harding, 1981). In zoos it is common to offer crickets or mealworm larvae to marmosets and tamarins (family Callithricidae) as a way of promoting foraging activity. Many small prosimian primates such as galagos (genus *Otolemur*), lorises (genus *Nycticebus*), and tarsiers (genus *Tarsius*) are also highly insectivorous; some tarsiers may refuse to eat other than insects or live prey. Unfortunately, insects used as food for captive primates are not nutritionally well balanced, being particularly low in calcium. Thus, supplementation of the insect source with calcium and other nutrients may be essential to prevent nutrient deficiencies in captive primates that ingest large quantities of insects. One method is to feed the insects themselves on high calcium diets, such that the insects have digestive tracts laden with calcium when they are consumed (Allen and Oftedal, 1989).

One additional diet specialization found among primates is gum feeding. Although many prosimians and callithricids feed opportunistically on gums and other plant exudates in their natural habitats, some species such as the pygmy marmoset (genus *Cebuella*) rely on these exudates as a principle foodstuff (Nash, 1986). Pygmy marmosets appear to have digestive responses that facilitate gum digestion, presumably by microbial activity since gums are believed to be resistant to mammalian digestive enzymes (Power, 1991). Gums are sometimes offered to these species in captivity, but these species appear to maintain good health even if they do not have access to plant exudates.

C. Stage of Life Cycle

The nutrient requirements in a colony of nonhuman primates within a species can be influenced by genetic and environmental factors. As with other mammalian species, the nutrient requirements of nonhuman primates vary with the stages of the life cycle, i.e., growth, reproduction, and maintenance. The decreased nutrient requirements for maintenance of mature and older nonhuman primates is not always recognized. Obesity can result if diets formulated for maximum growth or reproduction are fed to mature animals who are not in breeding programs. Obesity can most efficiently be controlled by feeding mature nonbreeding animals diets with a low caloric density or by limiting their food consumption.

D. Environmental Factors

Environmental factors that may influence the nutrient requirements of nonhuman primates include experimental or postsurgical stress, the type of housing (indoor or outdoor), and the season of year. When nonhuman primates are housed in groups it is important to ensure that the least dominant individuals in the hierarchy have access to food and water.

IV. REQUIRED NUTRIENTS

A. Energy

Daily energy intake must be sufficient to meet requirements for basal metabolism and activity (Lewis *et al.*, 1990). Dietary energy is provided by carbohydrates, fats, and proteins. Energy requirements may be affected by a variety of factors, such as species sex (in some species males typically grow more rapidly and achieve a larger body mass than females), age, physiological and/or reproductive status, health status, and environmental conditions. Energy requirements also decrease with the cessation of growth and maturation except for pregnant animals. Adult nonhuman primates require approximately 25–50% per unit of body weight of the energy required by infants of their respective species (Nicolosi and Hunt, 1979). Estimated energy requirements for several species of various ages are presented in Table I.

Energy is measured in foods as kilocalories, kilojoules, or energy per gram of diet and is expressed as amounts of energy required per kilogram of body weight per day. Energy utilization by the animal body is modified by various physiological expenditures. A diet contains combustible energy that is used by the animal. The potentially available energy is gross energy (GE) which can be liberated and measured by analytical bomb calorimetry (Lewis *et al.*, 1990). Because energy is lost in undigested diet and sloughed products of metabolic origin in the feces, this fecal energy (FE) loss must be considered. When GE is corrected for the loss of FE, the potentially available energy remaining is called digestible energy (DE). DE is the energy that has been absorbed by the animal for metabolism. Losses from this DE energy source include urinary (UE) and gaseous products of digestion (GPD). The energy remaining for metabolic benefit is then called metabolized energy (ME). ME is estimated to be 90–95% of the DE energy; DE is estimated to be 90–95% of GE energy. The energy terms, gross energy, digestible energy, and metabolized energy are referred to most frequently in describing energy requirements for various species. Manipulation of the dietary components, particularly carbohydrates and fats, can alter energy density in the diet to provide the potentially available calories for a prescribed level of intake. Unless specifically stated, gross energy values are assumed in the following discussion.

Current commercial nonhuman primate diets provide between 2.9 and 4.3 kcal/g (Purina, 1987). Experimental diets for marmosets and tamarins that were formulated to contain 3.9–4.2 kcal/g have helped to prevent "wasting marmoset syndrome (WMS)" (Clapp and Tardif, 1985). In a study of long-term

TABLE I

ESTIMATED ENERGY INTAKES OF LABORATORY SPECIES OF NONHUMAN PRIMATES

Species	Status	kcal/kg/day[a]	Reference
Baboons	Infant	<290[b]	Nicolosi and Hunt (1979)
	Adult (Fe)	53–72	NRC (1978)
Cebus	Infant	250–400[b]	NRC (1978); Nicolosi and Hunt (1979)
	Adult	100–150	Nicolosi and Hunt (1979)
Chimpanzee	Juvenile	100–120[b]	NRC (1978)
	Adult	50–60	NRC (1978)
Cynomolgus	Adult	72–120[b]	Nicolosi and Hunt (1979)
Gibbon	Adult	2194[c]	Chivers and Ramaekers (1986)
Marmoset	Adult	36.3[c]	Wirth and Buselmaier (1982)
Pig-tailed macaques	Adult	70–80	Kerr (1972)
Rhesus	Infant	270	Kerr (1972)
	1 year	190, 110[b]	Kerr (1972); Rana *et al.* (1984)
	Juvenile	130	Kerr (1972)
		110[b]	Chopra *et al.*, (1987)
	Adult	40–65	Kerr (1972); NRC (1978)
Squirrel monkey	Infant	300–600	NRC (1978); Nicolosi and Hunt (1979)
	Adult	100–300[b]	Nicolosi and Hunt (1979)
Tamarin	Adult	335[b]	Barnard *et al.* (1988)
New World	Infant	300–500	NRC (1978); Nicolosi and Hunt (1979)
Old World	Infant	200–300	NRC (1978); Nicolosi and Hunt (1979)

[a]Values in kilocalories of assumed gross energy per kilogram per day unless otherwise stated.

[b]Kilocalories of energy per kilogram of body weight per day.

[c]Kilocalories per day.

feeding of rhesus monkeys (*Macaca mulatta*), a commercial dry diet providing 3.5–4.2 kcal/g was compared to a liquid diet formulated for human use and containing 1.0–1.1 kcal/ml (Hansen and Jen, 1979). Both types of diets provided sufficient energy to sustain life, although *ad libitum* intakes varied for monkeys of different body weight ranges. Rhesus monkeys weighing 4–4.9 kg consumed 136 kcal/kg/day of the dry diet versus 107 kcal/kg/day when fed the liquid diet. At 5–5.9 kg body weight, monkeys consumed 6.6% less dry and 13.1% less of the liquid diet per kilogram of body weight. Heavier monkeys, between 6 and 8 kg body weight, consumed an average of 114 or 78 kcal/day of the dry or liquid diet, respectively. The energy intake for both diets decreased with increasing body weight and, presumably, age. A lower *ad libitum* intake for the liquid diet was attributed to a greater digestibility.

1. Obesity

High intake levels of energy-dense diets by immature animals may result in a rapid growth rate that potentially induces obesity later in life. The role of genetic predisposition is uncertain, however, as obesity has not been studied across multiple generations as it has in human research (Hansen, 1979) and often requires many years to become evident. Obesity has been observed in two species of Old World species, rhesus (*M. mulatta*) and pig-tailed macaques (*M. nemestrima*) (Ausman *et al.*, 1981). Obesity in seven *M. mulatta* males has been observed after laboratory habitation of 15 years and an *ad libitum* intake of a commercial diet containing 4.2 kcal/kg. All males demonstrated clinical symptoms associated with obesity (Ausman *et al.*, 1981). Among 873 rhesus monkeys (*M. mulatta*) in a 9-year study, obesity was observed only in females. Intragastric feeding of 600 to 1050 kcal/day to induce obesity among rhesus monkeys was successful in increasing body weights 20–67% above initial body weights (Walike *et al.*, 1977). There was, however, a strong tendency among the rhesus monkeys to return to normal body weight following cessation of the intragastric overfeeding, demonstrating a lack of the "intractable" obesity which occurs among humans (Hansen, 1979). Among the 33 rhesus (*M. nemestrina*) evaluated for obesity, only females became obese (Walike *et al.*, 1977). Among the obese females, there was a significant elevation of fasting insulin and abdominal adiposity was prevalent. The abdominal skinfold thickness proved to be a reliable index of obesity in these species.

Obesity has also been observed in maturing squirrel monkeys (*Saimiri sciureus*) fed semipurified diets. When squirrel monkeys were maintained on a commercial, natural ingredient diet that provided 4.2 kcal/g they remained relatively lean through adulthood (Ausman *et al.*, 1981). However, when fed purified diets containing 21–31% fat and 50–70% soluble carbohydrates (sucrose and dextrin) they became obese. By 4.5 years of age, obesity occurred in over 50% of monkeys of both sexes and approached 100% by 5.5–6.5 years of age. Carcass analyses of obese animals indicated that the fat content ranged from 18 to 40%. In contrast, cebus monkeys (*Cebus albifrons*) did not demonstrate a similar tendency toward obesity before or after sexual maturation when maintained and fed similarly in a parallel study (Ausman *et al.*, 1981).

2. Reproductive Stages and Newborns

Caloric intake is dependent on the menstrual cycle phase and the reproductive state in female monkeys. Periovulatory decreases in caloric intake have been reported for the baboon (*Papio* ssp.); among macaques (*Macaca mulatta*), caloric intake is reduced during the preovulatory period by about 44% as compared to all other phases. Estrogen has an apparent inhibitory influence on food intake during preovulation and the effect may persist during gestation (Kemnitz *et al.*, 1984). Energy restriction to 80% of *ad libitum* intake has been reported to promote energy efficiency among lactating baboons (*P. cynocephalus* and *P. anubis*). Further restriction to 60% of *ad libitum* intake stimulates a significant increase in body nutrient mobilization and a 20% reduction in milk production (Roberts

et al., 1985). Kemnitz *et al.* (1986) examined changes in food intake and the preference of sugars during menstruation and pregnancy among rhesus monkeys (*M. mulatta*). These authors found that intake varied inversely with circulating estrogen levels. Menstruating monkeys ate less during the follicular phase than during the luteal phase of their cycles. Intake was also lower during the last two trimesters of pregnancy when fetal growth and maternal weight gain are at their most rapid. This change in intake was attributed to a decreased energy expenditure during late pregnancy. However, pregnant monkeys exhibited an increase in consumption of sucrose solutions coincidental to the end of the first trimester. The preferential intake of sucrose solutions in increasing concentrations (0.02–0.5 *M*) strongly suggests an increased demand for energy when gut capacity is most limited because of rapid fetal growth. During the last two trimesters, more energy-dense diets, fed in smaller overall amounts, may be indicated.

Although newborns may suffer weight losses after the first 3 weeks of age, newborn rhesus monkeys follow a consistent growth pattern when they are well-nourished (Riopelle *et al.*, 1986). A 40% decrease of the required caloric intake can result in a subnormal growth rate among juvenile baboons. However, a 40% increase above caloric requirements results in a normal growth rate in the caloric-deficient baboons (Lewis *et al.*, 1986, 1989; Rutenberg and Coelho, 1988).

Young rhesus monkeys (*M. mulatta*) have been tested as a model for protein–calorie malnutrition. A 45-day trial, where the daily energy intake was limited to 55 kcal/kg body weight and protein to 2.42 g weight/day has been suggested as an appropriate model for malnutrition studies (Mehta *et al.*, 1980) and concomitant neurological effects (Rana *et al.*, 1984). In a similar study involving protein caloric malnutrition, Qazzaz *et al.* (1981) reported symptoms of kwashiorkor disease and suppressed immune responses in three baboons (*Papio* ssp.). When sucrose was used as the primary carbohydrate, Whitehead (1980) also reported the development of fatty livers among baboons that were manipulated as kwashiorkor disease models. Squirrel monkeys (*Saimiri* ssp.) demonstrated an adaptive response to protein–calorie malnutrition during a 6-week interval, and energy restriction did not seem to modify the protein requirement for weight maintenance (Ausman *et al.*, 1989).

In conclusion, there may be a marked difference in the requirements among individual animals within a species. This energy factor may have practical implications when animals are fed limited amounts of diet in an effort to control body weight.

B. Protein

Dietary protein is a source of nitrogen and amino acids that are required for growth and for synthesis of all structural and functional proteins. Amino acids are dietary essential when their carbon skeletons are not synthesized by the animal in quantities sufficient to meet requirements or are nonessential when they are endogenously synthesized in quantities to meet requirements from carbon and nitrogen precursors.

The nutritional value of dietary protein depends on both its quantity and quality. The quantity of dietary protein is reflected by the total nitrogen (N) content of the diet where protein = N $\times$ 6.25. Analysis of animal tissues shows that almost all of the N is present in the form of protein (Munro and Crim, 1980).

The most important determinants of the nutritional quality of a protein are the ratio and quantity of its constituent amino acids. Mitchell and Block (1946) demonstrated that protein synthesis in tissues occurs only when all required amino acids are provided simultaneously. Thus, an intracellular deficit of any amino acid could impair the utilization of the rest. From these observations, they proposed that the biologic value of a dietary protein would be determined by the essential amino acid present in the least concentration relative to the needs of the animal. This is called the "limiting amino acid" from which an "amino acid score" of protein quality can be calculated.

Mitchell (1964) suggested that the biologic value of a protein should be similar in different species because of the similarity in amino acid composition of their body proteins. However, Sammonds and Hegsted (1973) compared the efficiency of various proteins to promote growth in *Cebus albifrons* monkeys, humans, and rats and concluded that species differ in their ability to utilize protein. It is unknown whether such differences may occur within the various families of the order Primates.

1. Essential Amino Acid Requirements

Nutritional requirements for essential amino acids in the nonhuman primate have not been established (NRC, 1978). However, Waisman and Kerr (1970) reported the growth rates of infant rhesus monkeys fed diets with various amino acid compositions. Detrimental effects in nonhuman primates fed a deficiency (Wilgram *et al.*, 1958; Mann, 1966; Kerr *et al.*, 1969a; Neuringer *et al.*, 1980; Young *et al.*, 1989) and an excess (Waisman *et al.*, 1959; Kerr *et al.*, 1965; Kerr and Waisman, 1966a; Waisman and Kerr, 1970) of particular amino acids have been described. Flurer *et al.* (1987), Flurer and Zucker (1988), and Zucker and Flurer (1989) reported that adult *Callithrix jacchus* may require dietary arginine and histidine.

2. Protein Sources

Protein from animal sources is considered "high quality" compared to that from plants because it contains more favorable levels and ratios of the essential amino acids compared to protein from single plant sources. However, mixtures of plants whose proteins provide complementary ratios of amino acids can prevent syndromes of amino acid imbalance that result from single dietary sources of plant protein.

Kerr and Waisman (1970) fed infant rhesus monkeys (*M. mulatta*) two diets in which the protein was equally restricted but the protein source of one was soy and the other was casein.

Monkeys fed the diet containing soy protein had a significantly depressed rate of growth compared to those fed the diet containing casein.

Sammonds and Hegsted (1973) and Ausman *et al.* (1979) determined the protein requirement for the maintenance of young growing monkeys fed diets containing various sole sources of protein. These findings illustrated that animals fed a maintenance level of protein failed to grow but showed few other signs of protein deficiency. However, if the protein intake fell below the maintenance requirement, the monkeys became anorexic, resulting in increased morbidity due to protein deficiency (Sammonds and Hegsted, 1973).

Sammonds and Hegsted (1973) reported that the daily maintenance requirements of protein per kilogram of body weight for growing *Cebus albifrons* was 8.7 g when the protein source was wheat gluten, 2.8 g when wheat gluten was supplemented with its limiting amino acid, lysine, and 2.0 g when lactalbumin was the protein source. Ausman *et al.* (1986) evaluated the growth and nitrogen balance of cebus monkeys fed diets in which the protein sources were lactalbumin, casein, soy concentrate, soy isolate, or soy isolate plus methionine. Based on nitrogen balance, the potencies compared to lactalbumin were: casein, 62.2; soy concentrate, 69.0; soy isolate, 46.8; and soy isolate plus methionine, 90.7. When soy was the protein source, Zucker and Flurer (1989) also determined that essential amino acid supplementation improved nitrogen retention in common marmosets (*C. jacchus*).

Ausman *et al.* (1979) assessed the efficiency of protein utilization in infant squirrel monkeys (*Saimiri sciureus*) fed diets containing restricted amounts of lactalbumin, casein, and soy protein isolate as the sole protein source. Daily mean requirements for weight maintenance in young growing squirrel monkeys for lactalbumin, casein, and soy protein isolate were 3.43, 3.63, and 7.96 of protein/kg body weight, respectively.

These results, and those of Ausman *et al.* (1986), indicate that soy protein is half as efficient as lactalbumin for cebus and squirrel monkeys. The amino acid composition of protein thus has a major influence on the dietary protein requirements of nonhuman primates.

Soybean meal, a major source of protein in commercial monkey diets, has also been evaluated for its protein quality (Ausman *et al.*, 1979). Fitch *et al.* (1964) reported reduced gastrointestinal absorption of iron resulting in iron deficiency and anemia in rhesus monkeys fed a diet containing isolated soy protein. Anemia was also observed in infant squirrel monkeys fed a protein-deficient diet in which the protein source was the soy isolate (Ausman *et al.*, 1977), but not when lactalbumin or casein was the protein source. Flurer *et al.* (1985) examined the palatability, digestibility, influences on body weight, and consistency of feces in *C. jacchus* and *S. fuscicollis* fed diets with casein, lactalbumin, soy protein concentrate, and soybean meal. These protein sources provided similar results with the exception that soybean meal was approximately 10% less digestible.

Ausman *et al.* (1985a) and Robbins *et al.* (1988) reported that soy protein had no adverse effects on the pancreas of cebus monkeys or baboons, respectively. Studies of the influence of dietary soy protein on cholesterol metabolism in nonhuman primates have produced variable responses (Terpstra *et al.*, 1984; Barth *et al.*, 1984; Wolfe and Grace, 1987).

3. Methods of Estimating Protein Requirements

The nutritional quality of a protein is determined by its amino acid composition and the bioavailability of these constituent amino acids. Factors influencing bioavailability include the efficiency in which proteins and amino acids are utilized, species of animal, health of the animal, feed processing, and other dietary constituents (Kies, 1981). The most common approaches to measuring bioavailability are the factorial nitrogen balance procedures.

In factorial estimating, the total obligatory nitrogen loss from the body (i.e., through the urine, feces, and skin) is measured when the protein intake is very low. It is assumed that dietary proteins of high quality can replace these obligatory nitrogen losses on a gram for gram basis and that the nitrogen losses are thus equivalent to the minimum nitrogen requirement. However, Sammonds and Hegsted (1973), using a dose–response, slope–ratio assay to determine the protein requirements for young cebus monkeys, demonstrated that high quality proteins are not utilized with 100% efficiency in these primates. Furthermore, relative potencies of lower quality proteins for nonhuman primate growth are lower than predicted based on the results of rat assay procedures. These defects in factorial estimating result in an underestimation of protein requirements. Sammonds and Hegsted (1973) concluded that the assumptions made in the factorial method for determining protein requirements are not valid for the young monkey.

The nitrogen balance procedure involves determining directly the amount of dietary protein required to keep the animal in nitrogen equilibrium. However, this technique tends to overestimate true protein intake and underestimate N output (Munro and Crim, 1980). These defects result in a more apparent positive N balance than actually exists. The biologic value and the efficiency of utilization of the protein must also be considered when evaluating protein by nitrogen balance (Munro and Crim, 1980). Nitrogen balance is also influenced by energy intake; an increase in caloric intake improves N retention.

4. Estimated Protein Requirements

Protein requirements for nonhuman primates have been assessed in a variety of ways. Portman (1970) calculated the theoretical protein requirements of growing rhesus monkeys using the factorial approach. Allowing for maximum growth rates reported by Kerr and Waisman (1966b), and a protein digestibility and biologic value of 80%, it was estimated that the protein requirement was approximately 3.5 g/kg body weight.

Sammonds and Hegsted (1973) calculated that this value corresponds to 5.6 to 6.1% of the calories based on a caloric intake of 200–250 kcal/kg body weight. Using the same data it was estimated that the protein requirement for maintenance (no allowance for growth) was approximately 1.6 g/kg body weight or 2.5 to 2.7% of the calories as protein.

In the previously mentioned study, Sammonds and Hegsted (1973) further calculated regression formulas from a dose–response, slope–ratio assay to determine protein requirements for growth in young growing *Cebus albifrons* and *Cebus apella* monkeys. Protein requirements for maximum growth of cebus monkeys weighing from 400 to 1000 g and consuming a diet in which the protein source was lactalbumin ranged from 7 to 5.23% of the total calories (or 5.25–3.27 g protein/kg body weight), respectively. The results indicated that the requirement per unit weight for maximum growth is variable and dependent on the size of the monkey and the rate of growth.

Protein requirements for squirrel and cynomolgus monkeys (*Macaca fasicularis*) were also determined by the slope–ratio assay (Ausman *et al.*, 1979). Young, growing squirrel monkeys were fed diets in which the protein source was casein. It was determined that the protein requirements for optimal growth of monkeys weighing from 200 to 500 g ranged from 10.1 to 5.79% protein (as a percentage of total calories), respectively. However, De La Iglesia *et al.* (1967) reported that squirrel monkeys weighing between 500 and 900 g required a minimal protein requirement of 12.5% of total calories when the protein was soy. Ausman *et al.* (1985b) suggested that this difference was due to the potency of soy protein being only 50% of casein. It appears as if juvenile and adult squirrel monkeys can be maintained on diets containing a minimum of 6–7% protein of the total calories; however, doubling this amount allows for individual variance, stress, illness, and pregnancy (Ausman *et al.*, 1985b).

Ausman *et al.* (1979) also assessed the efficiency of protein utilization in cynomolgus monkeys fed diets containing restricted amounts of lactalbumin as the sole protein source. Extrapolating these data to macaques with a maximum growth of 5 g per day, and weighing from 500 g to 1 kg, these authors estimated a requirement of 6.6% or less of the calories as protein for maximum growth. This value agrees with an earlier estimate of a protein requirement of 6.5% as total calories for maximal growth in infant rhesus monkeys (Kerr *et al.*, 1970).

Sammonds and Hegsted (1973) and Ausman *et al.* (1979) fed diets containing high quality proteins and estimated the efficiency of protein utilization for cebus and cynomolgus monkeys growth to be 74 and 65%, respectively. However, a 30% efficiency for growth was reported for squirrel monkeys fed a diet containing high quality protein (Ausman *et al.*, 1979). The investigators speculated that the poor efficiency may be due to the poor utilization of dietary amino acids by the squirrel monkey (Ausman and Gallina, 1979; Ausman *et al.*, 1979).

Ausman and Hegsted (1980) used long-term feeding trials designed to study the protein requirements of adult cebus monkeys. Their protein requirement was estimated to be 7.5% of total calories (2 g protein/kg body weight/day) when lactalbumin was the protein source. Sammonds and Hegsted (1973) also showed that the protein requirement for maximal growth of young cebus monkeys ranged from 5.2 to 7.0% of the calories (5.2–3.3 protein/kg body weight/day). These results indicate that despite the significantly different requirements per unit body weight, the estimated protein concentration of the diet for maximal performance is similar for both growing and adult monkeys for this species due to a decrease in the requirement for protein and energy with age. In fact, there is a greater decrease in the energy requirement, since protein requirements expressed as a percentage of energy do not change or even may increase.

Riopelle *et al.* (1974) reported that the protein requirements for adult rhesus monkeys are similar to those for adult cebus monkeys. They fed adult rhesus monkeys diets containing graded levels of protein (casein) for 160 days. According to the plasma albumin concentrations, free nonessential to essential amino acid ratios, and the weights of the monkeys, the minimal daily protein requirement for adult rhesus monkeys was just above 2 g/kg body weight.

5. Callitrichid Protein Nutrition

Early observations of the protein needs of Callitrichidae indicated that they had a high quality protein requirement (Stellar, 1960; Deinhardt, 1970). In this primate family, an association between WMS and protein deficiency has been suggested (Barnard *et al.*, 1988; Brack and Rothe, 1981; Shimwell *et al.*, 1979).

Kirkwood *et al.* (1983) reported that *S. oedipus* suffering from chronic diarrhea and weight loss syndrome had a daily protein intake of 0.7 g/kg body weight. Flurer *et al.* (1983) used data collected from several species of callitrichids to suggest that a monkey weighing 400 g would consume 9.75 protein/kg body weight/day. A *Saguinas mystax* recovering from WMS was reported to consume 21.5 g protein/kg body weight (Barnard *et al.*, 1988). Flurer and Zucker (1985) reported that the serum albumin concentrations, body weights, and reproduction rates for *C. jacchus* and *S. fuscicollis* fed diets containing 12, 18, and 24% protein as lactalbumin were normal and similar. However, growing *S. fuscicollis* did not thrive as well as *C. jacchus* offspring when these monkeys were fed the diet containing 12% protein (5 g protein/kg body weight/day).

Flurer *et al.* (1988) and Zucker and Flurer (1989) used nitrogen balance studies to determine that 1.65 g protein/kg body weight$^{0.75}$ was required to maintain the N balance in *C. jacchus*. In other research, endogenous N excretion was estimated by the nitrogen balance technique and then the factorial method was used to estimate that the minimal protein requirement to monitor the N balance for *C. jacchus* was 1.96 g/kg body weight$^{0.75}$ (Flurer *et al.*, 1988). These values were used as the basis for estimating the appropriate daily protein requirement of

2.4–3.47 g protein/kg body weight$^{0.75}$ (Munro and Crim, 1980; Zucker and Flurer, 1989).

6. Protein and Calorie Relationship

The interrelationship between calorie and protein requirements has been examined in nonhuman primates. Sammonds and Hegsted (1978), Gallina *et al.* (1987), and Ausman *et al.* (1989) demonstrated that when infant cebus and squirrel monkeys were fed diets that were restricted in calories and protein to the level that growth was prevented, the protein deficiency was no more severe than monkeys restricted in protein alone. This conflicts with short-term studies using other animals and humans which indicated that energy restriction induces negative nitrogen balance (Calloway, 1981; Garza *et al.*, 1976; Calloway and Margen, 1971). Furthermore, it was reported that the protein deficiency in these monkeys resulted in an inefficient mechanism of energy utilization when compared to monkeys fed the control diet and when compared to monkeys whose diets were solely restricted in calories (Sammonds and Hegsted, 1978; Gallina *et al.*, 1987; Ausman *et al.*, 1989).

The relationship between nonprotein calorie intake and nitrogen intake on body weight, nitrogen balance, and serum protein response in malnourished *M. fascicularis*, being repleted by total parental nutrition, was investigated by Dempsey *et al.* (1988). The results indicate that nonprotein calorie intake and nitrogen intake are not equally important in affecting these parameters. Nonprotein calories significantly influence body weight, whereas nitrogen intake influences nitrogen balance and serum protein response.

7. Maternal, Juvenile, and Neonatal Protein Nutrition

Protein requirements during pregnancy and lactation in primates are not known. However, Oftedal (1984, 1991) reported that proteins in primate milk are presumably equivalent if not superior to reference proteins (casein and lactalbumin) that have been tested, and supply 7–22% of the energy. Oftedal (1991) suggested that the cost to the mother of producing milk that is high in protein (20–22% of energy) is indicative of high protein requirements of some nonhuman primate infants.

Many studies have evaluated the effects of protein deficiency in pregnant nonhuman primates and their offspring. Riopelle *et al.* (1975a, 1976a) and Riopelle and Shell (1978) fed pregnant rhesus monkeys isocaloric diets that provided 1, 2, or 4 g protein/kg body weight/day when the monkeys consumed 120 kcal/kg body weight/day. Similar amounts of diet were consumed relative to body weight. However, those monkeys fed the diet providing 4 g protein/kg body weight/day gained weight above that required by the fetus, but those fed the diet providing the lowest protein concentration had a postdelivery weight similar to preconception weights. Kohrs *et al.* (1976) and Portman *et al.* (1987) related very similar findings. An earlier study had reported that nonpregnant adult rhesus monkeys had lost weight consuming the same diets providing either 1 or 2 g protein/kg body weight/day (Riopelle *et al.*, 1974). These results suggest that the pregnant rhesus monkey has an increased metabolic efficiency during pregnancy (Riopelle *et al.*, 1975a).

Riopelle *et al.* (1975b, 1976a) and Kohrs *et al.* (1976) evaluated the effects of an inadequate protein intake during pregnancy on fetal and neonatal status in rhesus monkeys. The fetal and neonatal mortality rate ranged from 40 to 50% when the mothers were fed diets in which protein was 3.4% of the calories during pregnancy (Riopelle *et al.*, 1975b; Kohrs *et al.*, 1976). Similar values for fetal mortality due to protein deficiency in pregnant squirrel monkeys were reported by Manocha and Long (1977).

Kohrs *et al.* (1976) and Novy *et al.* (1981) reported that rhesus neonates born to mothers fed a low protein diet providing less than 0.5 g/kg body weight/day during pregnancy had a markedly reduced birth weight. However, Riopelle *et al.* (1975b, 1976a) and Cheek *et al.* (1976) showed that birth weights and prenatal linear growth of rhesus neonates were not affected when the maternal protein intake was reduced to 1 g/kg body weight/day. Subsequent studies examined the effects of maternal protein restriction on the growth of infant nonhuman primates. Riopelle and Favret (1977) reported that a daily maternal protein intake of 1 g/kg body weight did not affect weight, radius length, a weight/radius index, skeletal maturity, food intake, and food efficiency of infant monkeys studied for 1 year after birth. In another study, infants born to mothers fed a diet providing 0.44 g protein/kg body weight/day had significantly decreased body weights and head circumferences for 180 days after birth (Kohrs *et al.*, 1980).

Postnatal protein restriction in rhesus monkeys has been studied by Kerr *et al.* (1969a,b, 1970, 1975). Their results indicate that a diet providing 3% of the calories (2.0 g protein/kg body weight/day) could not support normal infant growth as judged by weight gain, head circumference, and body length. These values are higher than the maintenance requirement of 1.8% of the calories as protein estimated for growing cynomolgus monkeys (Ausman *et al.*, 1979). Riopelle *et al.* (1987) showed that during the perinatal growth phase females gained significantly more weight than males when fed a diet containing only 2.5% of the calories as protein. Cebus monkeys fed a diet for 20 weeks, in which 2.8% of the calories were protein, did not gain weight, but there was skeletal growth at low levels except for the skull (Fleagle *et al.*, 1975). These results are in agreement with data of Sammonds and Hegsted (1973), who reported a protein maintenance requirement of 2.8% of dietary calories for growing cebus monkeys weighing 400 g. Riopelle *et al.* (1975b) reported that there were no significant differences in serum total protein, albumin, or the albumin to globulin ratio between rhesus neonates from mothers fed either a high or a low protein diet. Neonates from mothers fed diets in which protein was 3.4% of the calories, however, had difficulty in maintaining their body temperatures when exposed to room temperature (Kohrs *et al.*, 1979).

The effect of maternal protein deprivation on the infant brain has been assessed in rhesus monkeys. Cheek *et al.* (1976) reported that a daily protein intake of 1.2 g/kg body weight in pregnant rhesus monkeys did not produce reductions in biochemical parameters used to assess growth in the cerebrum or cerebellum of the fetus. In another study, protein restriction (3.1% of dietary calories as protein) during gestation and early infancy resulted in reduced weights of the brain stem, cerebellum, and cerebrum in rhesus infants (Portman *et al.*, 1977). In a followup study, Portman *et al.* (1987) fed rhesus infants of protein-deprived mothers a diet that supplied a low concentration of protein (3.8% of calories as casein) from birth to 10 years of age. The results indicated that deficits in the central nervous system of the rhesus monkey observed at 1 month of age persisted but did not increase. However, there were no effects on brain size and composition in monkeys born to adequately nourished mothers and fed the low protein diet for 12 years. Riopelle *et al.* (1976b) evaluated early behavior in infant rhesus monkeys born to mothers fed protein-restricted diets during pregnancy. They determined that maternal protein intake had no effect on visual and auditory perception, feeding reflexes, and locomotion.

8. Indices of Protein Deficiency

There is no single biochemical procedure that can satisfactorily evaluate protein deficiency. However, the use of clinical and anthropometric assessments in conjunction with biochemical measurements can provide an adequate evaluation of protein nutritional status. Serum total protein and albumin levels have been used extensively to estimate protein nutritional status in nonhuman primates (Ramalingaswami, 1964; Sood *et al.*, 1965; Ordy *et al.*, 1966; Geist *et al.*, 1972; Kumar *et al.*, 1972; Riopelle *et al.*, 1974; Sammonds and Hegsted, 1978; Worthington *et al.*, 1979; Ausman and Hegsted, 1980; Lunn and Baker, 1984; Gallina *et al.*, 1987; Ausman *et al.*, 1989). Kumar *et al.* (1972) concluded that total serum protein and serum albumin concentrations were not diagnostic of early protein deficiency but instead reflect the severity of the malnutrition. In addition, Sauberlich *et al.* (1981) noted that these parameters can be influenced by other variables.

Serum amino acid composition has also been correlated with protein deficiency in nonhuman primates (Kumar *et al.*, 1972; Sammonds and Hegsted, 1978; Riopelle *et al.*, 1974; Enwonwu *et al.*, 1973; Worthington *et al.*, 1979). In general, with protein deficiency the essential amino acids threonine, valine, leucine, and isoleucine decreased whereas nonessential amino acids asparagine, glycine, and alanine increased. Acute changes in amino acid composition occurred during the first 3 to 4 weeks of protein deprivation in cynomolgus monkeys (Worthington *et al.*, 1979). Age as well as the level of available dietary protein determined the severity of the amino acid imbalance. Serum amino acid composition as an index for protein malnutrition is influenced by factors such as infections, diarrhea, calorie deficiency, and circadian rhythm (Sauberlich *et al.*, 1981).

Serum transferrin concentration is an index of severity of kwashiorkor (Sauberlich *et al.*, 1981). Serum transferrin did not significantly decrease until 12 weeks in *M. nemestrina* fed a protein-free diet (Kumar *et al.*, 1972). Thus, serum transferrin levels were not diagnostic of an early protein deficiency. However, transferrin concentrations were markedly affected in neonatal squirrel monkeys fed a diet providing 4.7 g protein/kg body weight/day, significantly decreasing initially but returning to normal levels after 16 weeks on the protein-deficient diet (Gallina *et al.*, 1987). It was concluded that the transferrin concentrations were influenced by the growth rate.

9. Pathology of Protein Deficiency

Investigators have observed many pathological effects due to protein malnutrition in nonhuman primates: alopecia (Coward and Whitehead, 1972; Worthington *et al.*, 1979; Ausman *et al.*, 1989), behavior changes (Coward and Whitehead, 1972; Geist *et al.*, 1972; Hill and Riopelle, 1974; Worthington *et al.*, 1979; Chamove, 1980), facial edema (Coward and Whitehead, 1972; Sammonds and Hegsted, 1978; Ausman *et al.*, 1989), retarded skeletal growth (Kerr *et al.*, 1973; Jha, 1980), anorexia (Worthington *et al.*, 1979; Kumar *et al.*, 1972; Enwonwu *et al.*, 1973), fatty liver (Wilgram *et al.*, 1958; Ramalingaswami, 1964; Deo *et al.*, 1965; Racela *et al.*, 1966; Ordy *et al.*, 1966; De La Iglesia *et al.*, 1967; Kerr *et al.*, 1970; Coward and Whitehead, 1972; Enwonwu *et al.*, 1977), increased cortisol concentrations (Enwonwu *et al.*, 1973; Ausman *et al.*, 1989), decreased plasma thyroxine and increased triiodothyronine concentrations (Portman *et al.*, 1985), anemia (Ghitis *et al.*, 1963; Sood *et al.*, 1965; Deo *et al.*, 1965; Kumar *et al.*, 1972; Sammonds and Hegsted, 1978), abnormal lipid metabolism (Ramalingaswami, 1964; Srinivasan *et al.*, 1977, 1979; Kerr and Helmuth, 1973; Sammonds and Hegsted, 1978; Ausman and Hegsted, 1980; Portman *et al.*, 1981, 1985, 1987), changes in enzyme activity (Ordy *et al.*, 1966; Kumar and Chase, 1972; Kumar *et al.*, 1972; Manocha and Olkowski, 1972), perturbation in major organs (Deo *et al.*, 1965; Racela *et al.*, 1966; Kerr *et al.*, 1973, 1976; Manocha and Olkowski, 1972; Enwonwu *et al.*, 1973; Portman *et al.*, 1977, 1987), perturbation of neural cytochemistry (Manocha and Olkowski, 1972, 1973; Manocha and Sharma, 1978; Enwonwu and Okolie, 1983), and abnormal iron metabolism (Sood *et al.*, 1965; De La Iglesia *et al.*, 1967; Enwonwu *et al.*, 1973, 1977).

In conclusion, there is a considerable amount of literature related to protein nutrition of the various nonhuman primates species. However, the foregoing review indicates that there are large gaps in the knowledge regarding the quantitative protein and amino acid requirements of practically all species involved in biomedical research. It also indicates that, as an area of study, protein nutrition in nonhuman primates is quite complex due to the large number of variables. Many of the issues concerning

the most appropriate diets for the well-being of captive nonhuman primate colonies are not likely to be resolved without additional data regarding their protein or amino acid requirements.

Protein requirements for mammalian species are generally estimated on the basis of crude protein. However, it should be recognized that physiologically amino acids are the required nutrients rather than crude protein. On this basis, the results of studies designed to determine the amino acid requirements of nonhuman primates would be more efficient than those designed to determine crude protein requirements.

Studies have indicated that dietary restriction may be beneficial to various species of laboratory animals. It is likely, therefore, that excessive dietary concentrations of protein could be as detrimental to nonhuman primates as deficient concentrations. This may be a major factor in the management of colonies of New World species that are traditionally fed diets with relatively high concentrations of crude protein.

C. Fats and Fatty Acids

Fat is an important component of diets because of its high caloric density (2.25 times the metabolizable energy of an equal amount of carbohydrate or protein). Fats are also included in diets as a source of the essential fatty acids, i.e., linoleic acid, linolenic acid, and arachidonic acid. Commercial monkey diets contain approximately 5% fat by weight for Old World species and 9% for New World species primates (Purina, 1987). Effects of dietary deficiencies of essential fatty acids include dryness and scaliness of skin with hair loss (Portman *et al.*, 1961), bone marrow hyperplasia, and anemia with concomitant vitamin E deficiency (NRC, 1978). Altered fatty acid ratios in red blood cells among preadolescent rhesus monkeys (*M. mulatta*) (Fitch *et al.*, 1961) and decreased concentrations of omega-3 (ω-3) fatty acids in red blood cells are also associated with deficiencies in essential fatty acids (Reisbick *et al.*, 1990). A dietary ω-3 fatty acid deficiency has also been associated with visual loss among infant rhesus monkeys (Neuringer *et al.*, 1984; Connor *et al.*, 1984).

The absence of essential fatty acids in the diet results in significantly reduced concentrations of linoleic acid in red blood cells and other tissues among *Cebus* monkeys (Portman *et al.*, 1961). Pre- and postnatal diets deficient in ω-3 essential fatty acids contributed to the development of low levels of docosahexaenoic acid in the brain, red blood cells, and plasma of young rhesus monkeys. Symptoms were alleviated by the addition of fish oil to the diet (Connor *et al.*, 1990). Deficiencies of linoleic acid in red blood cells have been corrected by treatment with 0.25 ethyl linoleate/kg body weight (Kerr, 1972). Linoleic acid supplied in the diet at 1–2% of the total caloric requirement apparently prevents essential fatty acid deficiency.

1. Concentration, Saturation, and Cholesterol

Fat concentration, degree of fat saturation, and cholesterol can affect lipid biochemistry and nonhuman primate pathology (Clarkson *et al.*, 1987; Getz *et al.*, 1987). Zarkins *et al.* (1990) reported aortic or arterial aneurysms formed in 13% of cynomolgus and 1% of rhesus monkeys fed an atherogenic diet for 16 to 24 months. In most species of monkeys, consumption of a diet containing 30–40% of calories as saturated fat and 0.5 mg of cholesterol/kcal (two times the average intake for North Americans) is sufficient to induce significant amounts of atherosclerosis in 1 or 2 years (Rudel *et al.*, 1983; Mather *et al.*, 1981). However, species differences in tolerance of fat type also exist. Cynomolgus (*M. fascicularis*) and African green (*C. aethiops*) monkeys were both fed diets containing 40% calories as butter (saturated fat) and as safflower oil (polyunsaturated fat) plus 0.8% cholesterol/kcal to induce atherosclerosis (Parks and Rudel, 1982). When polyunsaturated fat was fed, there was no lowering of whole plasma cholesterol or of low density lipoproteins (LDL) (Levy and Levy, 1981). The high density lipoprotein (HDL) was reduced significantly among the cynomolgus monkeys. In contrast, the African green monkeys displayed a significant decrease in the cholesterol concentration of whole plasma, LDL, and HDL when polyunsaturated oil was fed as compared to saturated oil. Rudel *et al.* (1983) concluded that polyunsaturated fat may be atherogenic rather than antiatherogenic for the cynomolgus. An inverse relationship between plasma carnitine, the mitochondrial carrier of long-chain fatty acids, and triglycerides was demonstrated among stump-tailed macaques (*M. arctoides*) (Bell *et al.*, 1982), and one pig-tailed monkey (*M. nemistrina*) fed a low-fat (5.2%) dry diet, but not a high-fat (15.9%) semipurified diet (Bell and DeLucia, 1984).

Cholesterol added to experimental diets at a concentration of 0.4 mg/kcal has been shown to affect serum lipids among *Macaca arctoides* (Bell *et al.*, 1982, 1983), *M. irus* fed 0.8% added cholesterol (Bell *et al.*, 1983), and squirrel monkeys fed 0.05–1.0 mg cholesterol per kcal (Ausman *et al.*, 1985b). A twofold increase in total plasma cholesterol levels was observed among pig-tailed macaques (*M. nemestrina*) fed 0.24 mg/kcal of added cholesterol (Willcox *et al.*, 1986). Squirrel monkeys (*Saimiri sciureus*) were fed a diet containing 24% butter and supplemented with 0.75 mg/kcal cholesterol. The plasma cholesterol among the monkeys increased threefold over their prefeeding concentration (Jones *et al.*, 1975). The cholesterol concentration in digestive, reproductive, and musculoskeletal tissues among squirrel monkeys fed 0.5% added cholesterol reached equilibration with the plasma cholesterol concentration by 2 months following the cholesterol dose (Raymond *et al.*, 1976).

There was no apparent difference between breast- and formula-fed infant baboons (*Papio* ssp.) until they were 16 weeks of age (Lewis *et al.*, 1988). At postweaning, both groups were fed diets containing 40% lard and 1.7 mg cholesterol/kcal. The breast-fed baboons at 5 years of age demonstrated a greater prevalence of atherosclerotic lesions than the formula-fed group, due primarily to higher VLDL and LDL-cholesterol/HDL-cholesterol lipoprotein ratios. Six- to 8-year-old baboons that had been breast fed demonstrated lower HDL-cholesterol concentrations and higher VLDL + LDL-cholesterol/HDL-cholesterol ratios, but no noted deleterious effects were seen

(Mott *et al.*, 1990). The authors of both papers concluded that breast vs formula feeding in infancy alters cholesterol metabolism and serum lipoprotein concentrations in adult baboons.

Dietary fat has been shown to affect serum lipids independently of added cholesterol (Howard, 1979; Hudson and McCraw, 1987). Mature male and female *M. nigra* were fed a low fat diet (2.5% corn oil) and two high fat diets (13.2% safflower or coconut oil in different ratios) to modify the polyunsaturated and saturated fat ratio. Serum triglycerides were lowest when the polyunsaturated fat was fed and were increased when the low fat diet was fed because of the greater carbohydrate concentration. The quantity of fat had a greater effect on serum cholesterol concentration than did the degree of saturation (Howard, 1979). Mott *et al.* (1985) showed that in the absence of dietary cholesterol, unsaturated fat increased the cholesterol content of gastrointestinal, liver, plasma, spleen, and lung tissues among young baboons (*Papio* ssp.) when compared to saturated dietary fat.

Age and sex can affect serum cholesterol levels among pigtailed macaques (*M. nemestrina*) fed 0.24 mg cholesterol/kcal (Willcox *et al.*, 1986). Although LDL-cholesterol was increased significantly among young, adult, and aged monkeys, the increase among the aged animals was significantly less than that of the young or adult groups. Among the youngest animals, HDL-cholesterol was significantly greater in the females; among the adults, HDL-cholesterol levels were equal among males and females.

Cardiac triglyceride and phospholipid concentrations of *M. fascicularis* were not affected by the type of dietary fat (Kramer and Sauer, 1983). Depot fat of nonhuman primates, however, has been shown to be influenced by the fat source when lard was added to the diet (Ackman *et al.*, 1981).

2. Sources of Fat

The source of oil also induces physiological variations. When the source of oil is a fish by-product, different responses have been noted. African green (*C. aethiops*) and cynomolgus monkeys (*M. fascicularis*) were infused with a high cholesterol diet (0.79 mg/kcal) to determine the composition of lymph duct lipoproteins. The cholesteryl ester content of all lymph lipoproteins was increased and the cholesterol transport rate was significantly increased (Klein and Rudel, 1983). One gram of cholesterol per day added to a diet with 16% butter increased the plasma cholesterol level 10 times above prefeeding level (Robbins and Branen, 1979). Cholesterol esters increased by nine times and free cholesterol by four times the initial levels. When 2% of the diet was cholesterol added to a 25% coconut diet, rhesus monkeys (*M. mulatta*) experienced a significant increase in cholesteryl esters of LDL, greater cholesteryl esters in the very high density lipoprotein component (VHDL), but no change in the HDL component (Soltys *et al.*, 1989). However, when fish oil was added to the diet to partially replace the coconut oil (12.5 and 18.8% fat replacements), plasma concentrations of all lipoprotein fractions were decreased. African green monkeys (*C. aethiops*) fed 11% fish oil diets have small LDL and less cholesteryl ester per LDL particle than monkeys fed lard at a similar concentration (Parks and Bullock, 1987; Parks *et al.*, 1989a). Fish oil has inhibited the development of atherosclerosis in rhesus monkeys (Davis *et al.*, 1987), and peanut oil has reduced diet-induced atherosclerosis in cynomolgus monkeys (*M. fascicularis*) (Alderson *et al.*, 1986). African green monkeys (*C. aethiops*) fed 11% dietary fish oil for 2.5 years exhibited significantly decreased hepatic triglyceride secretion (Parks *et al.*, 1990) and lower cholesterol and LDL in blood plasma (Parks *et al.*, 1989b). The serum lipid response to menhaden oil added to the diet (Ward and Clarkson, 1985) and various vegetable oils such as soy and safflower oils (Lin *et al.*, 1990) and coconut and corn oils (Nicolosi *et al.*, 1990) have also elicited varied physiological responses.

When premenarchial rhesus monkeys (*M. mulatta*) were fed diets in which fat provided 31 or 12% of total calories, 80% of the high-fat fed group exhibited early first ovulation compared to 40% of the low-fat fed group (Schwartz *et al.*, 1988). These reproductive changes were associated with significant differences in the endocrine profiles of the animals receiving more fat.

In conclusion, while specific dietary fat requirements have not been established for the various species of nonhuman primates used as models for human-related research, these findings should give direction to the eventual establishment of fat requirements, particularly when animals are to be maintained for life span studies and are subject to human-like maladies.

D. Carbohydrates and Fiber

1. Carbohydrates

Carbohydrates serve as the primary source of energy in laboratory animal diets. Sources of carbohydrates in commercial primate diets are starches, complex carbohydrates derived from cereal products, and soluble sugars such as sucrose and fructose (Purina, 1987).

Although no particular class of carbohydrate is required in primate diets, many herbivorous and omnivorous species display a preference for specific sugars which may be related to natural feeding habits. However, some specific sugars have been associated with particular clinical problems. Sucrose has potentiated the effect of NaCl in causing hypertension among adult spider monkeys (*Ateles* ssp.) (Srinivasin *et al.*, 1980; Karanja and McCarron, 1986) and caused a marked increase in serum total cholesterol (Srinivasin *et al.*, 1983) and triglyceride (Sharma *et al.*, 1986) levels among male cynomolgus monkeys (*M. fascicularis*). Adult male squirrel monkeys (*Saimiri* ssp.) have demonstrated a preference for sucrose (0.5 *M* solutions) over similar concentrations of maltose or polycose, a cornstarch-derived polymeric sugar (Sunderland and Sclafani, 1988). Bonnet monkeys (*Macaca radiata*) demonstrated equal preference for the three sugars but at lower concentrations (0.01 *M*). The bonnet monkeys may have taste receptors for starch-derived polysaccharides, whereas the squirrel monkey may lack such

receptors (Sunderland and Sclafani, 1988). The taste preference profiles of these sugars are consistent with natural food preferences of these monkey species.

Sucrose, maltose, glucose, and fructose were fed to male rhesus monkeys (*M. mulatta*) to examine the influence of sugar consumption on total caloric regulation. The intake of sugar solutions (particularly 0.2 and 0.4 *M* concentrations) was 2.5–6 times greater than that of plain water (Kemnitz and Neu, 1986). Marmosets (*Callithrix jacchus jacchus* and *C. j. penicillata*) were able to utilize 95% of the total dietary starch when maintained for 4 years on a standardized pelleted diet formulated to simulate a naturally selected diet (Wirth and Buselmaier, 1982).

2. Fiber

The separation of carbohydrate fractions of foods has long proven difficult. The crude fiber (CF) and nitrogen-free extract (NFE) analyses separate the relatively indigestible carbohydrate fraction from that which is well-digested. The NFE fraction, however, represents a heterogeneous moiety, and reagents used in the CF procedure solubilize considerable amounts of hemicellulose and associated lignin. CF determinations underestimate the true dietary fiber value and do not accurately reflect the readily digestible fractions. A more meaningful method of fiber fractionation which partitions fiber constituents into cellulose, hemicellulose and lignin fractions has been developed (Goering and Van Soest, 1970; Maynard *et al.*, 1979).

Dietary fiber, a mixture of many complex organic substances, is equivalent to the plant cell wall constituents in a diet. This fraction includes both water-soluble and water-insoluble substances, many of which are nonfibrous in nature. Some nutritionists define dietary fiber as the polysaccharides and associated plant cell wall substances that are resistant to mammalian digestive enzymes (Van Soest, 1983). These resistant components include mucins, gums, and mucilages which are not plant cell wall components but are involved with plant cell metabolism. These water-soluble substances have different physical actions in the gastrointestinal tract than does the neutral detergent fiber residue and are usually rapidly fermented by gut microorganisms. Pectin, a primary cell wall gel-forming substance, represents a complex group of polysaccharides in which D-galacturonic acid is a principal constituent. Pectin is rapidly fermented by gut microbes (Van Soest, 1983; Lanza and Butrum, 1986). Among African green monkeys (*C. aethiops*), the chronic intake of dietary psyllium husk, a gel-forming water-soluble fiber commonly used as a bulk laxative, results in greater colonic microbial metabolism as compared to dietary cellulose, a more indigestible fiber source (Costa *et al.*, 1989).

A requirement for dietary fiber has not been established for nonhuman primates. The crude fiber concentration in commercial nonhuman primate diets ranges from 2 to 8% (Krombach *et al.*, 1984; Purina, 1987; Kerr, 1972). The addition of 5–10% cellulose to provide necessary bulk in semipurified diets has been recommended (Newberne and Hayes, 1979). Early studies (Morin *et al.*, 1978) to determine the effect of dietary crude fiber on intestinal disorders and average recovery time among recently imported rhesus monkeys (*M. mulatta*) established that a research diet with 7% crude fiber was more successful at decreasing morbidity during a 60-day quarantine period than were diets containing 2.4 or 9.8% crude fiber or a commercially extruded diet containing 2.2% crude fiber. A 7% dietary fiber level may more closely approximate that of "wild-type" diets, thus decreasing the adaptation time in quarantine. Diets containing an adequate amount of fiber may reduce the risk of hair bolus obstruction of cecal contents among macaques (Clarke *et al.*, 1977). Experimental diets prepared for marmosets usually contain 4.2–10% fiber (Clapp and Tardif, 1985). Gorillas (*Gorilla gorilla*) are characterized as folivorous (Hladik, 1988), and in the wild consume considerable amounts of cellulosic plant materials, suggesting a possible benefit of higher fiber foods in diets of captive animals.

The concentration and type of dietary fiber should be evaluated in terms of animal health, particularly in long-term studies. Paulini *et al.* (1987) fed psyllium husk, a mucilaginous, gel-forming fiber source similar to pectin, and cellulose, a relatively indigestible fiber source, at 9.7% to African green monkeys (*C. aethiops* ssp. vervets) for 3.5 years. The addition of psyllium husk caused increased epithelial cell loss, hypertrophy of the jejunal–ileal muscle layer, and thinning of the colonic wall when compared to added cellulose. While both fiber sources were associated with cellular necrosis of the lamina propria as evidenced by scanning electron microscopy, animals appeared healthy and had no diarrhea or abnormal feces. However, because of the mucosal damage, care should be taken in recommending long-term feeding of gel-forming fiber. When low- and high-bran breads, based on wheat flour of 70 or 90% extraction, respectively, were fed to baboons (*Papio ursinus*) for 26 months, animals fed the low-bran bread developed more frequent and larger ileocecal mucosal microherniations (Kriek *et al.*, 1982). When bran diets containing a constant level of 10% fat were fed to baboons, cuboidal or squamous metaplasia of cecal mucosa were significantly more common among those baboons fed the low concentration of bran (Kriek and van Rensburg, 1980).

Fiber type and the mineral-binding properties of phytate (inositol hexaphosphate) in natural ingredient diets may influence the intestinal absorption of a number of essential elements (Kriek *et al.*, 1982). The fiber in bran may have a role in influencing the absorption of zinc, iron, calcium (Renan and van Rensburg, 1980), copper, sulfur, potassium, and nickel (Kriek *et al.*, 1982) in baboons (*P. ursinus*). Similarly, fiber type appeared to influence the absorption of copper among vervet monkeys (*C. aethiops pygerethrus*; Klevay *et al.*, 1981). However, in African green monkeys, the absorption of iron, zinc, and copper was within normal range when the monkeys were fed psyllium husk fiber (Paulini *et al.*, 1987). Energy and dry matter digestibility were depressed by the addition of wheat bran (up

to 6%) in diets fed to marmosets (*Callithrix jacchus*) and tamarins (*Saguinus fuscicollis*) (Krombach *et al.*, 1984).

Practical aspects of feeding high fiber diets should also be considered. Spiller (1981) fed psyllium husk fiber to pig-tailed monkeys (*M. nemestrina*) and measured increased fecal output with fecal moisture of 70–80% even at a lower fiber intake. Cellulose addition also resulted in an increased fecal output; however, fecal moisture was lower at 55–60%. Monkeys fed diets containing rice hull and oat straw increased fecal output with fecal moisture of 65%.

Commercially prepared biscuits fed to squirrel monkeys (*S. sciureus*) contained 2.3–3.5% fiber (Ausman *et al.*, 1985b). These products are frequently supplemented with a variety of succulent foods such as apples, bananas, oranges, and spinach which provide additional fiber (Lanza and Butrum, 1986). In semipurified diets, fiber has been derived from seeds, grains, wood, or vegetable pulp. Semipurified agar cake diets, containing 10% added fiber, have been considered adequate for squirrel monkeys. Although no fiber requirement has been established, in the wild this species ingests leaves, seeds, grains, nuts, and fruits (Ausman *et al.*, 1985b).

A natural ingredient diet formulated for chimpanzees (*Pan* ssp.) contains 3.5% crude fiber on an as-fed basis (NRC, 1978), whereas purified diets for both New and Old World species have included agar as a nonnutritive fiber source. Soluble fiber and insoluble fiber fractions, 11.5–16.8 and 4.2–6.5%, respectively, have also been used. A natural ingredient diet formulated as a standardized diet for marmosets (*Callithrix* ssp.) containing 3.0% crude fiber from wheat bran was successful in maintaining the health of adult marmosets for a 4-year period (Wirth and Buselmaier, 1982). Barnard *et al.* (1988) reported that "wasting marmoset syndrome" was reversed in *S. mystax* fed a natural ingredient diet containing 6% crude fiber from primarily beet pulp.

Effects of dietary fiber on serum and liver lipid levels and on atherosclerosis in nonhuman primates have been documented (Heine *et al.*, 1984; Kritchevsky *et al.*, 1986, 1988; Kritchevsky, 1987). The type of fiber affects lipid metabolism, e.g., bran and cellulose have little effect on cholesterol metabolism, whereas pectin has a marked influence. When vervet monkeys (*C. aethiops*) were fed semipurified diets containing 40% sucrose and 15% cellulose, wheat straw, or alfalfa for 23 weeks, the incidence of aortic sudanophilia was elevated (Kritchevsky *et al.*, 1981). Alfalfa and wheat straw lowered serum and liver cholesterol when compared to cellulose. Aortic sudanophilia in monkeys fed wheat straw was lower than among the monkeys fed cellulose. Since cellulose is more lipidemic and sudanophilic than wheat straw or alfalfa in vervet monkeys, the type of fiber present in semipurified diets can affect cholesterolemia and atherosclerosis. In a later study by Kritchevsky *et al.* (1988), a "Western-type" diet was developed to simulate a diet similar to diets of developed countries—42% of total calories as fat, 39.8% as carbohydrates, 14% as protein, and with either 10% apple pectin or 10% cellulose as fiber sources. Total serum cholesterol levels were elevated significantly in vervet monkeys after 34 weeks. Liver cholesterol levels were similar for both fiber-fed groups; liver triglycerides were lower in the cellulose-fed group.

The transit time through the gut may influence nutrient absorption. An increase in dietary fiber increases the rate of digesta passage through the gastrointestinal tract, thus reducing the time for digestion, as well as increasing fecal volume (Karanja and McCarron, 1986). Stimulation of bacterial growth, increased excretion of bacteria and their products, and increased desquamation of intestinal epithelial cells may be related to the depression of apparent digestibility of dry matter that results from the ingestion of high fiber diets (Krombach *et al.*, 1984). Viscous polysaccharides (guar and pectin) may delay transit through the small intestine, whereas more indigestible bran and cellulose may slightly increase the rate of passage. The viscous polysaccharides also delay the absorption of glucose from the small intestine. When chimpanzees (*P. troglodytes*) were fed diets containing either 34 or 14% neutral detergent fiber, ingesting the diet with the higher concentration of fiber decreased transit time and fiber digestibility (Milton and Demment, 1988). African green monkeys (*C. aethiops*) fed 9.7% dietary cellulose had a digesta transit time from mouth to anus of 2 days as compared to a 5-day transit when fed equal amount of psyllium husk (Paulini *et al.*, 1988). Although zinc absorption was similar among monkeys fed both fiber sources, the psyllium diet produced an apparent increase in copper absorption, but a decrease in serum copper concentrations. Liver and kidney copper were not affected by the fiber source. Transit time through the colon is decreased by high stool weight and water content. Fiber particles possess a water-binding capacity and thus can be effective in treating mild but not severe constipation.

In summary, dietary requirements for carbohydrates and crude fiber for most laboratory animal species are not well defined. Nonhuman primates are similar in this regard. It appears that there is a considerable amount of variation in the requirements for these dietary constituents among the various nonhuman primate species. Crude fiber appears to be a beneficial constituent of nonhuman primate diets, but adequate data are not available to base valid estimates of the amounts and quality required by various species require for optimal health.

E. Vitamins

Vitamins are a group of organic compounds that are essential in minute amounts for growth, maintenance, and reproduction. A concern regarding estimated vitamin requirements for different species of nonhuman primates is one that has not been adequately addressed experimentally. This raises concern about estimations of vitamin requirements for the order Primates as a whole. However, it appears that the current vitamin requirements are acceptable for satisfactory growth and reproduction in most nonhuman primate species used in biomedical research

(NRC, 1978). The question that remains is whether the vitamin requirements are sufficient during periods of stress such as disease states and experimentation.

1. Fat-Soluble Vitamins

a. VITAMIN A. This vitamin is essential for vision, growth, and tissue differentiation (Olson, 1984). The first signs of vitamin A deficiency in rhesus (*M. mulatta*) and cebus monkeys (*C. albifrons*) are loss of appetite, diarrhea, retarded growth, weakness, and an apparent increase in respiratory infections. In more severe stages, xerophthalmia, night blindness, corneal destruction, and retinal degeneration occur (O'Toole *et al.*, 1974; Hayes, 1974a; Rodger *et al.*, 1961; Ramalingaswami *et al.*, 1955). The vitamin A deficiency syndrome in rhesus and cebus monkeys was associated with reductions in plasma vitamin A from 26 to 10 μg/dl and from 20 to less than 5 μg/dl, respectively (O'Toole *et al.*, 1974; Hayes, 1974a). Other investigators have reported plasma vitamin A concentrations in healthy nonhuman primates that ranged from 8 to 140 μg/dl (McGuire *et al.*, 1989; Baly *et al.*, 1984; Cornwell and Boots, 1981; O'Toole *et al.*, 1974). Many factors influence plasma vitamin A concentration such as protein–calorie deficiency, ingestion of toxic substances, infestation with parasites, and other dietary nutrient concentrations (Olson, 1984). Baly *et al.* (1984) demonstrated that there is an interrelationship among zinc, vitamin A, and retinol-binding protein in rhesus monkeys. An interrelationship between vitamin E and vitamin A has been observed in nonhuman primates (Hayes, 1974a; Meydani *et al.*, 1983).

O'Toole *et al.* (1974) studied the effect of vitamin A deficiency on the reproductive performance of rhesus monkeys. The results indicated that the female monkeys were able to maintain regular menstrual cycles; 60% became pregnant and 40% gave birth to viable offspring. One male rhesus monkey became infertile after being fed the vitamin A-deficient diet for 9 months. Infant monkeys in this study developed vitamin A deficiency more rapidly than the adults. Vitamin A deficiency also resulted in xerophthalmia.

Vitamin A requirements are difficult to determine since they are dependent on age, sex, rate of growth, physical activity, caloric intake, stress, and other dietary nutrient concentrations (Olson, 1984). The NRC (1978) recommends 10,000–15,000 IU vitamin A/kg of diet. However, the vitamin A concentrations of commercial nonhuman primate diets range from 20,000 to 30,000 IU vitamin A/kg of diet and appear to support normal growth, reproductive efficiency, and good health. As a rule, hypervitaminosis A symptoms only appear at doses 10 to 50 times higher than the recommended dose (Olson, 1984). Principal signs of chronic vitamin A toxicity include skin dryness and pigmentation, alopecia, anorexia, weakness, leukopenia, hypoplastic anemia, enlarged liver and spleen, hepatocellular damage, bleeding lips and gums, stiffness in joints, and pruritus. Macapinlac and Olson (1981) showed that the LD_{50} for acute hypervitaminosis A in cynomolgus monkeys (*M. fascicularis*) was very high (168 mg/kg of body weight).

b. VITAMIN D. Vitamin D represents a group of fat-soluble steroids that have antirachitic activity. Vitamin D, calcitonin, and parathyroid hormone function to maintain calcium and phosphorous homeostasis. Ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3) are the two most prominent compounds. Ergocalciferol is derived from ultraviolet irradiation of ergosterol, a plant sterol, and cholecalciferol is produced by ultraviolet irradiation of 7-dehydrocholesterol which is found in the skin (Miller and Norman, 1984).

There is a history of skeletal disease related to vitamin D deficiency in captive nonhuman primates (Fiennes, 1974). Early evidence indicated that vitamin D_3 was much more effective than vitamin D_2 in preventing the development of metabolic bone diseases in Platyrrhines (New World primates) compared to Catarrhines (Old World primates) (Stare *et al.*, 1963; Hampton *et al.*, 1966; Lehner *et al.*, 1967; Hunt *et al.*, 1967a, 1969). Hunt *et al.* (1967b) demonstrated in cebus monkeys that vitamin D_3 promoted the intestinal absorption of radiolabeled calcium but that vitamin D_2 had a negligible effect on calcium absorption.

Investigations have centered on the difference of vitamin D metabolism between Platyrrhines and Catarrhines. The results of these studies suggest that the Platyrrhines have a target cell resistance to vitamin D (Edelstein, 1974; Shinki *et al.*, 1983; Adams *et al.*, 1985a,b; Takahashi *et al.*, 1985; Liberman *et al.*, 1985; Yamaguchi *et al.*, 1986; Marx *et al.*, 1989). However, a study of the relationship between end organ resistance and specific uptake with bioeffectiveness of 1,25-$(OH)_2$-D_3 in cultured skin cells from Platyrrhine and Catarrhine donors showed that among the Platyrrhines the 1,25-$(OH)_2$-D_3 phenotype of the species *A. trivergatus* is more closely related to the Catarrhine phenotype (Adams and Gacad, 1988).

The NRC (1978) committee recommends 2000 IU D_3/kg of diet for nonhuman primates, and this concentration has been suggested to be the optimal dietary level for Callithricidae (Flurer and Zucker, 1987). However, commercial nonhuman primate diets contain between 2000 and 9000 IU D_3/kg of diet. It has been demonstrated that squirrel monkeys (*S. scuireus*) and Callithricidae can be fed diets containing up to 10,000 IU D_3/kg of diet without adverse effects (Lehner *et al.*, 1967; Flurer and Zucker, 1987). However, studies indicate that rhesus monkeys, prosimians, and owl monkeys may be susceptible to vitamin D_3 toxicity at the higher concentrations found in commercial nonhuman primate diets (Hunt *et al.*, 1972; Gray *et al.*, 1982; Arnaud *et al.*, 1985; Vieth *et al.*, 1987; Marx *et al.*, 1989).

c. VITAMIN E. This vitamin and the selenium-containing enzyme glutathione peroxidase act together as antioxidants to prevent lipid peroxidation of polyunsaturated fatty acids (PUFA) in cell membranes (Machlin, 1984). It has also been shown in nonhuman primates that there is an interrelationship between vitamin E and the proteins, coenzyme Q, and lecithin–

cholesterol acyltransferase (phosphatidylcholine–sterol *O*-acyl transferase) (Fitch *et al.*, 1965; Farley *et al.*, 1967; Micket *et al.*, 1975). A requirement for vitamin E has been demonstrated in *M. mulatta* (Mason and Telford, 1947; Dinning and Day, 1957; Bieri and Evarts, 1972), *M. fascicularis* and *C. albifrons* (Ausman and Hayes, 1974; Hayes, 1974b), *A. trivergatus* (Meydani *et al.*, 1983), and *C. jacchus* (McIntosh *et al.*, 1987).

Many factors can affect vitamin E status, such as age (infants have lower blood tocopherol concentrations than adults), malabsorption disorders, protein–calorie malnutrition, other dietary nutrient concentrations, drugs, and heavy metals (Machlin, 1984). An association between malabsorption syndromes and vitamin E deficiency has been reported in colonies of *S. labiatus* and *C. jacchus* (Baskin *et al.*, 1983; Chalmers *et al.*, 1983).

Serum or plasma tocopherol concentrations are the most convenient and useful index of vitamin E status (Machlin, 1984). Serum tocopherol values in apparently healthy nonhuman primates ranged from 160 to 1500 g/dl, but in vitamin E-deficient nonhuman primates the values ranged from undetectable to 100 g/dl (Hayes, 1974a; Ausman and Hayes, 1974; Fitch *et al.*, 1980; Baskin *et al.*, 1983; Chalmers *et al.*, 1983; Meydani *et al.*, 1983; McIntosh *et al.*, 1987; McGuire *et al.*, 1989). Since plasma tocopherol concentrations are highly correlated with total lipid concentration, it has been suggested that a ratio of tocopherol to total lipid concentrations might more accurately reflect tissue levels and therefore vitamin E status (Horwitt *et al.*, 1972; Farrell *et al.*, 1978; Desai *et al.*, 1980).

Clinical signs of vitamin E deficiency in nonhuman primates include muscular dystrophy, anemia, anorexia, weight loss, muscle weakness, creatinuria, respiratory distress, retinal degeneration, and reduced resistance to infection (Fitch and Dinning, 1963; Fitch, 1968; Bieri and Evarts, 1972; Hayes, 1974a,b; Ausman and Hayes, 1974; Fitch *et al.*, 1980; Brady *et al.*, 1982; Walsh *et al.*, 1982; McIntosh *et al.*, 1987). Tocopherol deprivation for more than 12 months was required before vitamin E deficiency signs were demonstrated. Hayes (1974a) reported, however, that retinal and testicular degeneration occurred prior to the other clinical parameters. Anemia due to vitamin E deficiency in nonhuman primates has been reported to be either normocytic or macrocytic and normochromic with limited reticulocytosis (Fitch *et al.*, 1965; Ausman and Hayes, 1974; Hayes, 1974b; Fitch *et al.*, 1980; Wixson and Griffith, 1986). Fitch *et al.* (1980) demonstrated that the limited reticulocytosis is due to dyserythropoeisis. The anemia varies with the species of nonhuman primate. For example, deficient cebus monkeys developed a hemolytic anemia in 1 year whereas hemolytic anemia did not appear in deficient cynomolgus monkeys for 2 years and was much less severe (Hayes, 1974a). It has also been shown that certain karyotypes of *Aotus trivergatus* have a vitamin E-deficient-like hemolytic anemia that is ameliorated by an intramuscular injection of selenium and vitamin E (Sehgal *et al.*, 1980; Béland *et al.*, 1981; Meydani *et al.*, 1983).

Vitamin E requirements are difficult to define since dietary PUFA, selenium, and sulfur amino acids can influence the requirement for the vitamin. Several studies have shown that dietary PUFA exacerbates vitamin E deficiency in nonhuman primates, with cebus monkeys (*C. albifrons* and *C. apella*) being more adversely affected than cynomolgus monkeys (Fitch and Dinning, 1963; Hayes, 1974a; Ausman and Hayes, 1974). Fitch and Dinning (1963) suggested that the vitamin E requirement for *M. mulatta* fed a diet containing 3% cod liver oil and 8% lard was 2.9 mg/kg body weight and for those fed a fat-deficient diet it would be 0.7 mg/kg body weight. More recent data suggest a minimal daily requirement of 0.16 mg/kg body weight for *C. albifrons* and 0.10 mg/kg body weight/day for *M. fascicularis* (Ausman and Hayes, 1974). In agreement with Ausman and Hayes (1974), Bieri and Evarts (1972) reported that 0.36–0.72 mg of $^{(+)}$α-tocopherol acetate/g of dietary linoleic acid was adequate for *M. mulatta*. Evidence also exists that there can be strain differences in vitamin E requirements within a species, as in *A. trivergatus* (Béland *et al.*, 1981).

d. VITAMIN K. Vitamin K is a cofactor for glutamyl carboxylase, an enzyme involved in the activation of several blood-clotting factors (Suttie, 1984). Metta and Gopalan (1963) and Hill *et al.* (1964) fed vitamin K-deficient diets to *M. mulatta* without producing a hemorrhagic condition. However, Hill *et al.* (1964) produced an increase in prothrombin clotting time that was prevented by 0.1 μg vitamin K/kg body weight/day. The preferred means of measuring vitamin K status is plasma concentration of the vitamin K-dependent clotting factors or plasma vitamin K_1 and a 24-hr excretion of γ-carboxyglutamic acid (Suttie, 1984).

The dietary requirement for vitamin K is difficult to determine since monkeys have the ability to utilize vitamin K synthesized by intestinal bacteria. Suttie (1984) reported that *M. mulatta* required 2 μg vitamin K/kg body weight/day. Factors influencing vitamin K status include age (neonates and the elderly are at risk of vitamin K deficiency), protracted antibiotic treatment, large amounts of vitamin E and malabsorption due to obstructive jaundice, pancreatic insufficiency, steatorrhea, or chronic diarrhea (Suttie, 1984).

2. Water-Soluble Vitamins

a. THIAMIN. Thiamin is the precursor of thiamin pyrophosphate (TPP), a coenzyme required for the decarboxylation of α-keto acids and for transketolase. Thus, thiamin is involved in the metabolism of carbohydrates, fats, and proteins (Gubler, 1984). Also, TPP has a specific function in neurophysiology separate from its coenzyme capacity (Plaitakis *et al.*, 1982).

Leblond and Chaulin-Serviniere (1942), Waisman and McCall (1944), Rinehart *et al.* (1948, 1949), and Blank *et al.* (1975) have reported thiamin deficiency signs in rhesus monkeys that included weight loss, anorexia, apathy, cachexia, muscle weakness, ataxia, convulsions, and cardiac insufficiency. Rinehart *et al.* (1948) reported that the first signs of thiamin deficiency in *M. mulatta* appeared within 2 weeks, and Blank *et al.* (1975)

observed neurological signs between 7 and 10 weeks after initiating the study. A rapid development of deficiency signs also occurs with other water-soluble vitamins and is due to the high turnover rate of the vitamin in the body and the reduced capability for storage in tissues (Gubler, 1984).

Thiamin requirements for animals are affected by several factors, such as other dietary nutrient concentrations (carbohydrates, fat, Mg^{2+}, Ca^{2+}, vitamin B_6, vitamin B_{12}, and folate), size of the animal, intestinal microflora, genetic factors, and age of the animal (Zieve, 1969; Sebrell and Harris, 1973; Howard *et al.*, 1974; Gubler *et al.*, 1976; Kimura and Itokawa, 1977; Nishino and Itokawa, 1977; Gubler, 1984). Waisman and McCall (1944) measured the blood pyruvate concentration and Rinehart *et al.* (1948) used the blood thiamin concentration, but both groups estimated the minimal daily thiamin requirement of 15.5 μg/kg body weight. However, these methods of thiamin assessment have been determined to be inadequate measures of thiamin status (Gubler, 1984). The most reliable method to measure thiamin status is erythrocyte transketolase activity (Sauberlich *et al.*, 1974). The range of 15–30 mg/kg body weight per day to avoid deficiency signs was recommended by the NRC study committee (NRC, 1978).

b. RIBOFLAVIN. This vitamin is essential for the synthesis of the coenzymes, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) which act as intermediaries in the transfer of electrons in biological oxidation–reduction reactions. The FAD and FMN flavoproteins are required for the metabolism of carbohydrates, fats, and proteins. These enzymes are also essential for the conversion of pyridoxine and folic acid into their coenzyme forms (Cooperman and Lopez, 1984). This vitamin occurs in foods as FAD, FMN, and free riboflavin. All three can satisfy the requirement for this vitamin (Cooperman and Lopez, 1984).

Riboflavin deficiency signs in nonhuman primates include weight loss, seborrheic dermatitis, gingivitis, diarrhea, normocytic hypochromic anemia, alopecia, ataxia, abnormal tryptophan metabolism, adrenal cortical hemorrhage, and sudden death (Day *et al.*, 1935; Waisman, 1944; Cooperman *et al.*, 1945; Rinehart *et al.*, 1948; Mann *et al.*, 1952a,b; Greenberg and Moon, 1963; Foy *et al.*, 1964, 1972; Foy and Kondi, 1968; Greenberg, 1970). However, unlike other water-soluble vitamins, riboflavin deficiency develops rather slowly; weight loss occurred after 6 to 8 weeks and dermatological changes appeared after 2 to 6 months. An interaction among riboflavin, pyridoxine, and folic acid that occurs in most other animals was also observed in riboflavin-deficient monkeys (Foy *et al.*, 1964).

Greenberg and Moon (1963) reported plasma and erythrocyte riboflavin values for deficient rhesus monkeys as 3.2 and 21.5 μg/dl, respectively. The plasma and erythrocyte values for control monkeys were 7.6 and 30 μg/dl, respectively. Plasma and urinary excretion values reflect current intake, whereas erythrocyte values are more indicative of riboflavin status. Measurement of erythrocyte glutathione reductase is the most sensitive method to measure riboflavin status (Cooperman and Lopez, 1984). Factors affecting riboflavin status include antibiotics, thyroid disease, diabetes, heavy metals, and intestinal parasites.

The dietary requirement for riboflavin varies among members of the same species due to differences in body size and age (Cooperman and Lopez, 1984). Cooperman *et al.* (1945) and Mann *et al.* (1952b) reported the minimum daily requirement for both cebus and rhesus monkeys to be about 25–30 μg/kg of body weight.

c. NIACIN. Niacin is the precursor to coenzymes nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate which are hydrogen carriers esential in the release of energy from carbohydrate, fat, and protein (Hankes, 1984). The amino acid tryptophan is a precursor of niacin and can meet the requirements for niacin, with approximately 60 mg of tryptophan equal to 1 mg of niacin (Rao and Gopalan, 1984).

The signs of niacin deficiency in rhesus monkeys include weight loss, alopecia, anemia, diarrhea, chronic atrophic gastritis, atrophic necrotizing enterocolitis, reduced concentrations of erythrocyte pyridine nucleotides, and abnormal skin pigmentation on the face, phalangeal joints, and perineum (Cooperman *et al.*, 1946a; Tappan *et al.*, 1952; Belavady *et al.*, 1968; Belavady and Rao, 1973). Niacin status is influenced by deficiencies of carbohydrate, protein, or B complex vitamins. The niacin deficiency syndromes are complicated further by the biological availability of the bound forms of niacin in foods, as well as factors influencing the conversion of tryptophan into niacin (Hankes, 1984).

Niacin deficiency in the rhesus monkey was reversed by a daily intraperitoneal injection of between 10 and 40 mg of niacin/animal (Tappan *et al.*, 1952; Belavady *et al.*, 1968; Belavady and Rao, 1973). Tappan *et al.* (1952) reported that 10–35 mg niacin/monkey/week or 1–2 g of tryptophan/week reversed deficiency. This high requirement for tryptophan indicates an inadequate conversion to nicotinic acid which may be due to the protein, energy, pyridoxine, riboflavin, or tryptophan status of the monkeys (Rao and Gopalan, 1984). The suggested niacin requirement for nonhuman primates ranges from 50 to 110 mg/kg of the diet (Tappan *et al.*, 1952; NRC, 1978; Hankes, 1984).

d. VITAMIN B_6. The naturally occurring physiologically active forms of vitamin B_6 (*pyridoxine*) are pyridoxal and pyridoxamine. The principal functions of pyridoxine and its analogs are the synthesis and metabolism of amino acids (Driskell, 1984).

Pyridoxine deficiency signs in nonhuman primates include dermatitis, poor growth, weight loss, hyperirritability, hypochromic microcytic anemia, fatty liver, hepatic necrosis and cirrhosis, oxaluria, apathy, ataxia, neuropathy, and convulsions (McCall *et al.*, 1946; Rinehart and Greenberg, 1949, 1951, 1956; Poppen *et al.*, 1952; Mushett and Emerson, 1956; Victor

and Adams, 1956; Greenberg *et al.*, 1958; Emerson *et al.*, 1960; Gershoff, 1964; Greenberg, 1964; Wizgird *et al.*, 1965; Mann, 1968). Arteriosclerosis was reported in pyridoxine-deficient *M. mulatta* but is not a common deficiency sign in humans and most other animals including the cebus monkey (Rinehart and Greenberg, 1949, 1951, 1956; Mushett and Emerson, 1956; Greenberg *et al.*, 1958; Greenberg, 1964; Mann, 1968; Driskell, 1984). Vitamin B_6 is involved in lipid metabolism but the mechanism is unknown (Driskell, 1984). The relationship among dietary fat, pyridoxine, and atherosclerosis in *M. mulatta* has been investigated (Greenberg and Moon, 1959, 1961; Greenberg, 1964). The results indicated that a combined essential fatty acid (EFA) and pyridoxine deficiency did increase the severity of the vascular lesions when compared to lesions from pyridoxine-deficient monkeys. However, EFA deficiency alone did not increase the severity of vascular lesions when compared to those from pyridoxine-deficient monkeys. Emerson *et al.* (1960) reported that total plasma lipids were increased when higher concentrations of pyridoxine were fed to *M. mulatta*.

Pyridoxine status is affected by drugs, uremia, liver disease, stress, pregnancy, dietary protein, niacin, and riboflavin (Mann, 1968; Manning and Clarkson, 1971; Driskell, 1984). Isoniazid, a drug used for prophylaxis and therapy of tuberculosis, has been reported to be a pyridoxine antagonist (Levy *et al.*, 1967). However, Manning and Clarkson (1971) did not observe decreased serum vitamin B_6 concentrations in *M. mulatta* receiving 0.06% dietary isoniazid and 2.4 mg of pyridoxine per day for 11 months. In another study, increased dietary protein exacerbated the severity of the pyridoxine deficiency in cebus monkeys (Mann, 1968). The influence of these factors on pyridoxine status may explain the large variation in suggested daily pyridoxine requirements for nonhuman primates: 50–500 μg/kg of body weight (McCall *et al.*, 1946; Emerson *et al.*, 1960; Rinehart and Greenberg, 1956).

Methods for the nutritional assessment of pyridoxine include blood pyridoxine concentration, tryptophan load test, measurement of erythrocyte alanine aminotransferase activity, and plasma pyridoxal 5-phosphate concentration (Driskell, 1984). Serum pyridoxine concentrations for deficient and control *M. mulatta* were 3 and 11–24 μg/dl, respectively (Greenberg *et al.*, 1958; Manning and Clarkson, 1971).

e. BIOTIN. Biotin is a cofactor for enzymes involved in lipogenesis and gluconeogenesis (Bonjour, 1984). Lease *et al.* (1937), Waisman and Elvehjem (1943), and Waisman *et al.* (1945) have reported on biotin deficiency in nonhuman primates. Waisman *et al.* (1945) induced an acute biotin deficiency by feeding *M. mulatta* a diet containing 10% dried eggwhite (avidin) or 1% sulfaguanidine or sulfasuxidine. The deficiency signs were scaly dermatitis on the hands and feet, alopecia, depigmentation of the hair, and increased susceptibility to infection. The deficiency was corrected by administering 10 μg biotin/kg body weight/day to a 2-g monkey. The estimated nutritional requirement for biotin in nonhuman primates is 100 μg/kg of diet and is based on data from Waisman *et al.* (1945). However, this estimate was determined without considering the potential enteric synthesis of biotin by intestinal microorganisms, intestinal flora which require biotin, or the bioavailability of biotin in the diet, all of which can influence biotin status (Bonjour, 1984).

f. PANTOTHENIC ACID. This is incorporated into coenzyme A and acyl-carrier protein. As part of these proteins it is involved in the release of energy from carbohydrates, fats, and proteins and also in the synthesis of amino acids, fatty acids, sterols, and steroid hormones (Fox, 1984). McCall *et al.* (1946) reported that the signs of pantothenic acid deficiency in *M. mulatta* are growth retardation, anemia, diarrhea, cachexia, depigmentation of the hair, alopecia, and ataxia. These investigators observed only partial improvement in the deficient monkeys given 1 to 2 mg of calcium pantothenate/kg of body weight/day. However, Greenberg (1970) reported a much better response when 3 mg of calcium pantothenate/day was administered to deficient monkeys. This disparity may be due to other dietary factors that influence pantothenic acid status such as fat, protein, methionine, vitamin B_{12}, ascorbic acid, biotin, and folic acid (Fox, 1984).

g. VITAMIN C. At one time vitamin C deficiency was one of the most frequently diagnosed nutrient deficiencies in captive nonhuman primates (NRC, 1978; Nicolosi and Hunt, 1979). A possible explanation for this was improper storage of the diet resulting in destruction of vitamin C. Kessler (1980) and Demaray *et al.* (1978) determined that exposure of the diet to heat and humidity, and then soaking the diet in water prior to feeding destroyed most of the vitamin C, resulting in deficiency signs in squirrel monkeys. The most significant characteristic of L-ascorbic acid is its oxidation to dehydro-L-ascorbic acid to form a redox system. These compounds are active antiscorbutic agents. Physiological functions of ascorbic acid such as steroid hydroxylations and collagen metabolism are primarily dependent on its reducing properties and its role as an electron carrier (Jaffe, 1984). Elliot *et al.* (1966) demonstrated that the prosimians *Tupaia glis* (tree shrew) and *Nycticebus coucang* (slow loris) have the enzyme L-gulonolactone oxidase that is required to synthesize ascorbic acid. However, all other primates investigated, including *Papio ursinus* (DeKlerk *et al.* 1973b), *C. aethiops* (DeKlerk *et al.*, 1973a), *M. mulatta* (Turnbull *et al.*, 1981), *M. fasicularis* (Tillotson and O'Connor, 1980), *S. sciureus* (Kessler, 1980), *C. fatuellus* (Shaw, 1949), *C. jacchus*, and *S. fuscicollis* (Flurer and Zucker, 1989), require a dietary source of vitamin C. Vitamin C deficiency signs have been reported to be gingival hemorrhage, loose teeth, periodontal disease, depressed polymorphonuclear leukocyte activity, subperiosteal hemorrhage, normocytic normochromic anemia, epiphyseal fractures, and exophthalmus (Banerjee and Bal, 1959; Lehner *et al.*, 1968; Demaray *et al.*, 1978; Alvares *et al.*, 1981). Cephalhematoma appears to be the diagnostic sign of vitamin

C deficiency in young squirrel monkeys (Blackwell *et al.*, 1974; Demaray *et al.*, 1978; Kessler, 1980).

Vitamin C status is most commonly determined by measuring the vitamin C concentration of serum, whole blood, erythrocytes, and leukocytes (Jaffe, 1984). Turnbull *et al.* (1981) showed in rhesus monkeys that when plasma values of ascorbic acid were greater than 1–3 μg/ml, any blood component may serve as an index of vitamin C status. However, below this level leukocyte vitamin C concentration was the only reliable indicator.

Daily vitamin C requirements for nonhuman primates have been estimated to range from 1 to 25 mg/kg of body weight (Day, 1944; Shaw *et al.*, 1945; Soloveva *et al.*, 1966; Lehner *et al.*, 1968; DeKlerk *et al.*, 1973a,b; Baker *et al.*, 1975; Flurer and Zucker, 1989). Environmental stress influences vitamin C status, according to Jaffe (1984), and it has been suggested that this may explain the large variation in estimated vitamin C requirements in nonhuman primates (Tillotson and O'Connor, 1980, 1981). Flurer and Zucker (1989) suggested that the *S. fuscicollis* has a higher requirement than *C. jacchus*. Tillotson and O'Connor (1980) determined that juvenile monkeys require more vitamin C (6 mg/kg of body weight/day) than mature monkeys (3 mg/kg of body weight/day).

h. VITAMIN B_{12}. Cobalamin (vitamin B_{12}) is essential for the normal function of cells of the gastrointestinal tract, bone marrow, and nervous tissue (Krause and Mahan, 1984). Cobalamin is involved in folic acid and nucleic acid metabolism and is necessary for the transfer of methyl groups during the synthesis of methionine from homocysteine (Ellenbogen, 1984).

Unlike humans, nonhuman primates do not readily manifest overt hematological and neurological abnormalities from a vitamin B_{12} deficiency (Wilson and Pitney, 1955; Das Gupta *et al.*, 1955; Krohn *et al.*, 1963; Oxnard, 1964; Oxnard *et al.*, 1970; Greenberg, 1970; Siddons, 1974a; Kark *et al.*, 1974). This inability to induce the usual manifestations of vitamin B_{12} deficiency may be due to high stores of the vitamin in tissues, the difficulty in formulating a vitamin B_{12}-deficient diet, or in controlling intake of the vitamin from extraneous sources such as coprophagia and/or ingestion of insects (Agamanolis *et al.*, 1976; Ellenbogen, 1984). It is also possible that vitamin B_{12} status in nonhuman primates is influenced by intestinal bacteria. Oxnard (1969) suggested that the high serum vitamin B_{12} concentration in langur monkeys is due to absorption of the vitamin from intestinal bacteria in their rumen-like upper gastrointestinal tract.

Recently captured nonhuman primates had serum vitamin B_{12} concentrations that averaged 300 pg/ml but fell rapidly for 3 months and ranged from 20 to 60 pg/ml after 1 to 2 years in captivity (Krohn *et al.*, 1963; Oxnard, 1964; Flinn and Oxnard, 1966; Oxnard *et al.*, 1970). Diarrhea and antibiotics are possible factors contributing to the rapid decrease in serum vitamin B_{12} concentration in nonhuman primates during the first few months in captivity (Siddons, 1974a; Ellenbogen, 1984). Oxnard (1964) and Flinn and Oxnard (1966) reported that pregnancy also exacerbated vitamin B_{12} deficiency in rhesus monkeys.

Oxnard (1966, 1969), Oxnard and Smith (1966), Oxnard *et al.* (1967, 1969, 1970), Hind (1970), and Torres *et al.* (1971) have reported neurological abnormalities in nonhuman primates with low serum concentrations of vitamin B_{12}. However, because of the lack of a controlled diet it was not possible to show an exact causal relationship. Following those studies, more controlled long-term investigations showed neuropathologic changes that included demyelination and axon loss in the optic nerve and white matter of the spinal cord (Kark *et al.*, 1974; Agamanolis *et al.*, 1976; Chester *et al.*, 1980). These pathologic findings were accompanied by clinical neurologic changes such as optic atrophy, visual impairment, and spastic paralysis of hindlimbs. The clinical and pathologic changes occurred 33 to 45 months after institution of the vitamin B_{12}-deficient diet and in the absence of hematologic abnormalities.

Vitamin B_{12} requirements for nonhuman primates have not been established, but Flinn and Oxnard (1966) reported a weight gain in rhesus monkeys receiving 500 μg of vitamin B_{12}/week. Huser and Beard (1969) and Beard and Huser (1970) reported normal to high serum vitamin B_{12} values in Pongidae receiving 15.7 μg/day and Catarrhines receiving 3.5–6 μg/day. However, these serum vitamin B_{12} concentrations were measured by the isotope dilution method which can result in artificially high values (Ellenbogen, 1984).

i. FOLIC ACID. Day *et al.* (1935) and Langston *et al.* (1938) reported a nutrient deficiency in *M. mulatta* resulting in stomatitis, gingivitis, diarrhea, weight loss, lethargy, leukopenia, and anemia that could be corrected by a nutrient found in liver extract or yeast. The unknown nutrient was designated vitamin M and was subsequently identified as folic acid. Folate coenzymes function as donors of methyl units in the synthesis of cellular DNA, RNA, and proteins (Brody *et al.*, 1984). These processes are involved in cell growth and proliferation (Mohanty and Das, 1982).

Dietary folate deficiency has been produced in three Platyrrhine species, *C. jacchus* (Dreizen *et al.*, 1970), *S. scuireus* (Rasmussen *et al.*, 1979), and *C. albifrons* (Rasmussen *et al.*, 1982), and in two Catarrhine species, *M. mulatta* (Day *et al.*, 1935; Langston *et al.*, 1938; Waisman and Elvehjem, 1943; Cooperman *et al.*, 1946b; Day and Totter, 1947) and *P. cynocephalus* (Siddons, 1974b). The most common folic acid deficiency signs observed in nonhuman primates have been weight loss, leukopenia, and anemia. Mucous membrane lesions in the mouth have been observed in rhesus monkeys (Langston *et al.*, 1938), marmosets (Dreizen *et al.*, 1970), and baboons (Siddons, 1974b), as well as in the gastrointestinal tract of marmosets (Dreizen *et al.*, 1970) and cebus monkeys (Rasmussen *et al.*, 1982). Other signs of folic acid deficiency in nonhuman primates include alopecia, diarrhea, asthenia, scaly dermatitis, and depressed immunocompetence (Langston *et al.*, 1938; Waisman and Elvehjem, 1943; Dreizen *et al.*, 1970; Siddons, 1974b; Ras-

mussen *et al.*, 1979, 1982). The relationship among folic acid deficiency, cell growth, and cell proliferation in rhesus monkeys was investigated by Mohanty and Das (1982). They reported that folate-deficient rhesus monkeys had an increased number of atresic and cystic ovarian follicles with depletion of granulosa cells. Megablastosis, multinucleation, and impairment of orderly proliferation and maturation were observed in the cervicovaginal epithelium.

Folic acid status is most commonly assessed by the direct measurement of serum and erythrocyte folic acid. Serum folic acid reflects recent dietary intake whereas erythrocyte folic acid indicates body folic acid stores at the time of erythropoiesis. Huser and Beard (1969) reported serum and erythrocyte folic acid values in several species of nonhuman primates that ranged from 10 to 60 ng/ml and from 27 to 230 ng/ml, respectively. Their data show normal serum folate values but the erythrocyte values in some species indicated low body stores.

Many factors affect folic acid status such as pregnancy, lactation, growth, hemolytic anemia, malabsorption diseases, genetic diseases, and vitamin B_{12} deficiency. Gyr *et al.* (1974) showed that absorption of folic acid in *E. patas* decreased when there was a protein deficiency. Rasmussen *et al.* (1980) reported that supplementation of pregnant squirrel monkeys with folic acid improved hematologic and folate status, maternal weight gain, and infant birth weight. Blocker *et al.* (1989) showed that neonatal folate status in cebus monkeys was related to the dietary folate intake of the mother during pregnancy and lactation.

The minimum daily folic acid requirement for young growing rhesus monkeys has been estimated to be 40 μg/kg body weight (Cooperman *et al.*, 1946b; Day and Trotter, 1947). Rasmussen *et al.* (1979, 1980) estimated that, for maintenance of body weight, squirrel monkeys require 28 μg of total folacin/kg body weight/day. However, more than 75 μg of total folacin/kg of body weight was needed daily to assure growth, normal hematological parameters, and bone marrow cytology. Rassmussen *et al.* (1980) also suggested that diets fed to squirrel monkeys maintained for breeding should provide at least 450 μg of total dietary folate/kg of body weight/day to allow for 25% availability of the food forms of the vitamin as is assumed for man. The minimal daily folic acid requirement for adequate growth and normal hematological parameters in cebus monkeys has been estimated to be between 45 and 75 μg/kg of body weight (Rasmussen *et al.*, 1982).

j. CHOLINE. Choline serves as a source of labile methyl groups for the biosynthesis of methylated compounds such as acetylcholine, phosphatidylcholine, and sphingomyelin (Chan, 1984). Choline is synthesized by many animal species (Friedman and Frenkel, 1972) but there are chemical and clinical conditions and disorders which require dietary choline supplementation (Barbeau *et al.*, 1979). Choline deficiency signs in the nonhuman primates include decreased blood lipids, alopecia, weight loss, lassitude, elevation of liver enzymes, fatty livers, portal cirrhosis, and portal hypertension (Wilgram *et al.*, 1958; Hoffbauer and Zaki, 1965; Gaisford and Zuidema, 1965; Cueto *et al.*, 1967; Ruebner *et al.*, 1969; Rutherford *et al.*, 1969). All of these studies used diets low in protein or methionine and high in fat or cholesterol. These alterations in the diet are only a few of the factors known to aggravate choline deficiency (Wilson, 1979). However, due to these alterations in the diet a direct cause/effect relationship between choline deficiency and the reported deficiency signs cannot be established.

k. CARNITINE. This is important in the regulation of fatty acid metabolism but is nonessential for most mammals (Chan, 1984). However, it has been reported that there are clinical conditions and disorders related to impaired lipid disorders in which carnitine supplementation may be beneficial (Mitchell, 1978; Frenkel and McGary, 1980; Borum, 1981). These conditions include liver impairment, diabetes, kwashiorkor, hypopituitarism, adrenal insufficiency, pregnancy, physical exertion, and fasting disorders. It has also been demonstrated that neonates have a critical need for carnitine since they lack the full biosynthetic capacity for it (Borum, 1981).

Bell *et al.* (1979) showed that the effect of dietary cholesterol on serum carnitine in *M. mulatta, M. speciosa, C. aethiops,* and *S. sciureus* was different for each species. The level of serum carnitine observed in the *M. mulatta, M. speciosa,* and *C. aethiops* was similar to levels in humans (40–60 nm/ml). Bell *et al.* (1982) also investigated the influence of dietary fat on the serum carnitine concentration in *M. arctoides.* Total serum carnitine was reduced when the monkeys were fed a high fat diet (45% of calories from fat) but it was increased when the monkeys were fed a low fat diet (10% of calories from fat) or during fasting. The results suggest that fatty acid oxidation was increased during the high fat diet, thus requiring more carnitine to be synthesized by the body.

F. Minerals

1. Introduction

Naturally occurring mineral elements are termed "essential" if they have been demonstrated to have a metabolic role. The essential mineral elements are commonly grouped as "major" and "trace" elements based on their relative concentration in animal bodies. The use of radio- and stable isotope techniques has advanced the knowledge of mineral nutrition in recent years and many more minerals are now recognized as essential to long-term health and well-being. However, the identification of a mineral element as essential does not lead directly to an estimation of a recommended intake. For nonhuman primates, the diversity of dietary niches has resulted in a very limited understanding of mineral element requirements. Serious over- or undernutrition of specific minerals often produces symptoms of faulty metabolism (Kerr, 1972). It is difficult to discern, however, subtle signs that may be attributed to marginal mineral

adequacy which may result in long-term deficits and serious health problems or morbidity.

The salt mixes of Hegsted *et al.* (1941), Hawk *et al.* (1949), and the modification of the Hegsted salt by Hayes *et al.* (1975) have proved satisfactory for use in laboratory-formulated diets with both Old and New World monkeys. Much of the existing knowledge about mineral requirements in nonhuman primates is based on the report of Greenberg (1970) in which he monitored the formula consumption of young health rhesus monkeys and reported the average mineral and vitamin intakes.

2. Major Elements

a. CALCIUM. Calcium (Ca) is an important constituent of bones and teeth and is the mineral that is found with the highest concentration in animal bodies. Calcium is also involved in membrane transport; in the metabolism of skeletal tissues, contractile muscles, and blood coagulation; and as a cocatalyst in many enzyme systems. Harris *et al.* (1961) compared Ca^{45} excretion in rhesus monkeys after intravenous injection, stomach intubation, and dietary intake. This and other investigations of calcium metabolism in rhesus monkeys have indicated that 150 mg Ca/kg body weight/day for a growing 3-kg monkey is adequate for maintenance. However, Griffiths *et al.* (1975) produced osteoporosis in rhesus monkeys with a calcium concentration of 0.15% of diet by weight. (This amounted to 150 mg Ca/kg of body weight per day for 2- to 3-kg animals and 60–75 mg Ca/kg body weight per day for older animals.) Garruto *et al.* (1989) reported motor neuron pathology in juvenile cynomolgus monkeys maintained on low calcium diets (0.32% Ca) for approximately 3.5 years. The addition of aluminum and manganese to the diet led to signs of abnormal absorption and deposition of these elements in neurons. The authors concluded that a chronic dietary deficiency of calcium could induce metabolic abnormalities that lead to abnormal absorption and deposition of toxic elements resulting in motor neuron pathology. Considering the known effects of other minerals and nutrient concentrations on calcium retention, the National Research Council Committee report (NRC, 1978) suggested a calcium concentration of 0.5% of the diet by weight for adequate maintenance of nonhuman primates. Commercial diets commonly include 1% dietary calcium. This range (0.5–1% diet) should be adequate if other nutrients such as phosphorus and vitamin D are supplied in adequate levels. Similar to human milk, the calcium concentration in rhesus monkey milk increases slightly during the course of lactation, from 350 ± 26 μg/ml in colostrum to approximately 400 μg/ml in mature milk (Lönnerdal *et al.*, 1984). Calcium and phosphorus metabolism is closely tied to insufficient or excess amounts of vitamin D and its metabolites. This issue of adequate vitamin D is particularly important for nonhuman primates in indoor enclosures. (See discussion of this point in a previous section of this chapter.)

It has been demonstrated in humans that protein intake in excess of requirements leads to a reduction in calcium retention through increased calciuria (Allen, 1982; Heaney *et al.*, 1982). As the protein level is increased, independent of the calcium level, excretion of calcium in the urine increases whereas fecal excretion of calcium remains constant (Johnson *et al.*, 1970; Margen *et al.*, 1974). There is no evidence of increased calcium absorption, thus the increase in calciuria can lead to a negative calcium balance as a result of the increased glomerular filtration rate and decreased tubular calcium reabsorption (Johnson *et al.*, 1970; Spencer *et al.*, 1978a; Allen *et al.*, 1979; Schuette *et al.*, 1980; Block *et al.*, 1980). Similar studies have not been conducted in nonhuman primates. Consideration should thus be given to a calcium balance when determinations of dietary protein are made for captive primates.

Restricted physical activity in humans has also been demonstrated to result in a decreased efficiency of dietary calcium utilization and loss of bone mass (Whedon and Shorr, 1957; Donaldson *et al.*, 1970; Vogel and Whittle, 1976; Aloia, 1981). In monkeys who experienced activity restriction, a significant increase in calcium excretion has been reported (Pyke *et al.*, 1968; Kazarian and von Gierke, 1969). Captive monkeys with severely limited exercise over long periods of time may be at risk for early development of calcium-related diseases such as osteoporosis and osteoarthritis (DeRousseau, 1985a,b; Pritzker *et al.*, 1985; Rothschild and Woods, 1992). There has been some controversy over the prevalence of osteoarthritis in nonhuman primates (Ford *et al.*, 1986; Jurmain, 1989; Chateauvert *et al.*, 1990; Sokoloff, 1990). In 1517 skeletons from 29 nonhuman primate species, Rothschild and Woods (1992) reported clear indications of well-defined osteoarthritis and calcium pyrophosphate deposition disease in relatively few individuals, 1.7 and 2.5, respectively. These authors concluded that osteoarthritis was significantly more common in artificially restrained specimens when compared with free-ranging specimens for both prosimian and nonprosimian Old World primates. The predominant elbow involvement of artificially restrained animals significantly differed from the predominant knee involvement of free-ranging specimens which further supports the contention that the environmental disparity was not merely a reflection of an increased life span. Dietary protein, vitamin D, and calcium/phosphorus levels thus bear careful consideration for animals with restricted activity over their life span.

b. PHOSPHORUS. Phosphorus is essential in bone growth and development. It also plays an important role in carbohydrate metabolism and occurs in phosphoproteins, nucleic acids, and phospholipids. Low intakes of dietary phosphorus in domestic livestock have been associated with low fertility and reduced milk production. Investigations of phosphorus requirements in nonhuman primates are limited. Mineral mixes of Hawk *et al.* (1949) and Hayes *et al.* (1975) appear to supply an adequate level of phosphorus (0.2% of the diet by weight) and, with the additional phosphorus available in most protein sources, result in

levels of 0.3–0.4% in the daily diet (NRC, 1978). Limited analysis of the phosphate concentration in rhesus monkey milk indicated an approximately two to one calcium to phosphorus ratio (Lönnerdal *et al.*, 1984). Research with fungal phytase preparations may allow increased utilization of plant phytate–phosphorus dietary formulations for nonhuman primates.

The ratio of calcium to phosphorus in an animal diet has long been monitored as a measure of dietary adequacy. Studies with humans indicate that variations in the calcium/phosphorus ratio have an influence on calcium utilization or bone except in rapidly growing animals (Spencer *et al.*, 1978b, 1986, 1988; Draper, 1979; NRC, 1989). In a long-term study (3–88 months) with a low dietary calcium/phosphorus ratio with *Cebus albifrons*, Anderson *et al.* (1977) also reported no demonstrable skeletal loss.

c. POTASSIUM. Potassium serves as the primary intracellular cation and is involved with cell membrane transport processes. Potassium also functions in carbohydrate metabolism and muscle excitability. The range of 0.24–1.09% potassium in the diet appears adequate (Hummer, 1970; NRC, 1978). The potassium concentration in rhesus monkey milk is slightly higher in colostrum (367 ± 33 μg/ml) than in milk collected after 30 days (260 ± 8 μg/ml) (Lönnerdal *et al.*, 1984).

d. SODIUM. Sodium is the primary extracellular cation and is important in determining the distribution of body water. Sodium is commonly present in sufficient quantities in a natural ingredient diet. Sodium loss due to vomiting or diarrhea in monkeys should be compensated by supplementation or administration of fluids containing electrolytes.

The influence of a high dietary salt (NaCl) intake on blood pressure has been studied in a number of nonhuman primate species. African green monkeys, spider monkeys, and hamadryas baboons respond to an increased NaCl intake (3–6% dietary NaCl) with elevated mean systolic and diastolic blood pressures, whereas rhesus monkeys in a similar study failed to show a blood pressure change with a 3% NaCl diet over a 6-week period (Cherchovitch *et al.*, 1976; Srinivasan *et al.*, 1980, 1984). Marked individual differences exist among animals in all studies; individuals with high initial values became hyperresponders and individuals with lower initial values exhibited elevated but relatively lower blood pressures with a heightened NaCl intake. Monkeys displayed dislike for the high sodium diets, and a decline in mean body weight initially accompanied each increase in dietary NaCl.

For standard dietary practices, diets containing 0.22–0.64% of sodium appear adequate for Old World primates (Hummer, 1970; NRC, 1978). The average sodium concentration of rhesus monkey milk decreases slightly after the first week of lactation from 171 ± 64 to 89.4 ± 8.6 μg/ml at approximately 1 month (Lönnerdal *et al.*, 1984).

e. CHLORINE. Chlorine is important in gastric secretion (in the form of hydrochloric acid) and is associated with sodium and potassium in osmosis. Optimal or minimum dietary intakes for chlorine have not been established for nonhuman primates. Growth retardation with no other major symptoms has been associated with feeding chlorine-deficient diets to rodents for limited time periods. Hummer (1970) fed baboons diets with a range of 0.27–0.62% chlorine without apparent difficulties. Based on these data and the amounts which are currently contained in commercial diets, the National Research Council Committee (NRC, 1978) recommends a daily chlorine intake of 0.2–0.5%.

f. SULFUR. Sulfur is contained in the vitamins biotin and thiamin as well as in proteins containing the amino acids cystine, cysteine, and methionine. A deficiency of sulfur is linked with protein or vitamin deficiencies and is usually not considered separately. As mentioned previously, reports indicate that the sulfur amino acid component of dietary proteins may be in part responsible for changes in renal handling of calcium in humans consuming a high protein diet.

g. MAGNESIUM. Magnesium is associated with calcium and phosphorus in the skeleton and functions as a major cofactor for enzymes. Dunn (1971) produced hypomagnesemia with clinical signs of hyperirritability after 3–4 weeks with a diet containing 3 mg of magnesium per 100 g of diet. A control diet containing 102 mg of magnesium per 100 g of diet prevented development of a deficiency state. Treatment with 0.48 mg/ml of magnesium (33% of the control value) stabilized the condition. For adult monkeys the present commercial dietary concentration of 0.12–0.2% appears adequate as no deficiency states have been reported. However, there are few reports of laboratory studies of magnesium nutriture in any nonhuman primate species. Magnesium concentrations in rhesus monkey colostrum are significantly higher than in mature milk (colostrum: 49.6 + 12.1 μg/ml; mature milk at 1 month: 32.9 + 3 μg/ml) (Lönnerdal *et al.*, 1984) and indicate the need for supplementation of this mineral in hand-reared rhesus monkeys.

3. Trace Elements

a. IRON. Iron forms an important component of hemoglobin, myoglobin, a number of enzymes, and the cytochrome system. Specific types of anemia result from different types of deficiencies. Diets containing protein only from soybeans produced a reduction of iron absorption in the gastrointestinal tract resulting in microcytic hypochromic anemia in rhesus monkeys (Fitch *et al.*, 1964). An iron deficiency manifested by microcytic anemia with an elevated total iron-binding capacity, decreased liver iron stores, but normal intestinal iron absorption was demonstrated in outdoor corral-housed juvenile rhesus monkeys. The animals were repleted using their standard commercial diet when they were moved indoors. The authors concluded that items foraged by the animals in their outdoor enclosure interfered with the bioavailability of the iron in the standard commercial diet. The biological availability of trace

elements can be limited by many factors (Rosenberg and Solomons, 1982; Ashmead and Christy, 1985), and for nonhuman primates housed outdoors the plant material, soil, and environmental metallic contaminants must be considered potential inhibitors and interactants with the bioavailable nutrients provided in commercial diets.

An iron overload also produces deleterious effects including liver damage in rhesus monkeys (Nath *et al.*, 1972). Smith (1982) studied iron binding and transferrin polymorphism in rhesus monkeys and reported differential iron-binding capacities and serum total iron levels depending on genotype. He noted that these genetic differences may be associated with differential rates of fertility and infant survival in rhesus monkeys. In addition, he noted that the mean total iron-binding capacity for rhesus monkeys (401.2 μg/dl) was higher than normal humans (350 μg/dl) whereas serum total iron values were lower for the monkeys (96.3 μg/dl) than for humans (120 μg/dl) which corresponds to a species difference in saturation indices. Based on a study using a low iron diet and an iron overload induced with injections of iron dextran followed by iron chelation therapy, cebus monkeys have been suggested as models for humans in the evaluation of new iron chelators (Wolfe *et al.*, 1989).

Although no specific dietary levels have been identified for nonhuman primates, a diet containing 0.018% iron appears adequate for growing and adult rhesus monkeys (NRC, 1978). Iron in rhesus monkey milk dropped from 1.76 ± 0.84 to 1.15 ± 0.19 μg/ml over the first month of lactation (Lönnerdal *et al.*, 1984). The authors further commented that approximately 50% of the iron in monkey milk was bound to lactoferrin which has been hypothesized to account for the high availability of iron in human milk when compared with other mammalian species such as cow's milk. The biological availability of iron in formulas fed to hand-reared monkeys should thus be reviewed.

b. COBALT. Cobalt (Co) deficiency in domestic livestock was identified as early as 1807 and was characterized as "pining" or a "wasting" syndrome with listlessness and emaciation. This syndrome was associated with low concentrations of Co in soil and pastures of specific geographic areas and was prevented by feeding small amounts of cobalt (McDonald *et al.*, 1973). Cobalt is an essential element in the production of vitamin B_{12} by microorganisms in ruminant animals and is involved in enzyme reactions in ruminants and nonruminants alike. There is no recommended level of dietary Co for nonhuman primates. Traces found in natural-based diets appear sufficient to avoid deficiency.

c. COPPER. Copper (Cu) is essential for hemoglobin formation; it plays an important role in many enzyme systems and appears to be involved in cholesterol regulation. Demonstration of the teratogenicity of Cu deficiency during gestation has been shown in wild and experimental rodents (Keen and Hurley, 1976; Hurley and Keen, 1979; Hurley *et al.*, 1980). Copper deficiency also results in changes in tissue superoxide dismutase activity which leads to varying degrees of cell damage depending on other constituents in the diet (Fields *et al.*, 1984). Immunocompetence is also impaired in Cu-deficient animals (Prohaska and Lukasewycz, 1981). Symptoms of Cu deficiency include anemia, poor growth, bone disorders, change in pigmentation of the hair, gastrointestinal disturbances, and lesions in the spinal cord. Similar to humans, diet-induced copper deficiency is rare in nonhuman primates but the optimal level has yet to be determined (NRC, 1978). Commercial diets that typically contain from 12 to 20 mg of Cu/kg of diet appear adequate. Rhesus monkey milk averaged 3.04 μg copper per ml of milk during the first days of lactation and dropped rapidly to 0.46 μg/ml at 36 days postpartum (Lönnerdal *et al.*, 1984).

Similar to other minerals, the copper nutriture of an animal is closely linked to other dietary components. In particular, elevated intakes of dietary zinc have been shown to reduce copper retention in humans (Fischer *et al.*, 1984; Festa *et al.*, 1985). Housing monkeys in galvanized caging and thereby exposing them to high levels of zinc has been implicated as a causative factor in the "fading infant" syndrome in rhesus monkeys who exhibited significantly lowered plasma copper levels when compared with controls housed in stainless-steel cages (Stevens *et al.*, 1977; Obeck, 1978). Cynomolgus monkeys maintained on a low copper diet shared a 20% drop in serum copper levels and a similar but more rapid decline in ceruloplasmin activity in a study designed to study the interaction of ascorbic acid on copper metabolism and cholesterol (Milne *et al.*, 1981). Other potential nutrient–nutrient interactions urge careful consideration of the total dietary composition when fruit or treat supplements are used on a regular basis to augment a commercial or formulated diet that is provided to captive primates.

d. IODINE. Thyroid hormone synthesis necessitates adequate levels of dietary iodine. Iodine uptake in fetal and pregnant monkeys has been the only area of investigation of this mineral (Schultz *et al.*, 1965; Pickering, 1968). Mano *et al.* (1985) reported an iodine deficiency in marmosets (*Callithrix jacchus jacchus*), demonstrated by reduced plasma thyroxine and increased thryoid-stimulating hormone, using a diet that provided 0.3 μg iodine per day; control diets provided 7.6 μg iodine per day. Requirements have not been identified for specific nonhuman primate species, but diets with 2 ppm iodine have kept monkeys in good health (NRC, 1978).

e. MANGANESE. Enzyme activation is a major function that has been identified for manganese (Mn) in mammals, primarily in activation of phosphate transferases and decarboxylases. Deficiency signs in animals include poor reproductive success, poor growth, and leg deformities (Underwood, 1977). The dietary requirements for Mn have not been identified for nonhuman primates. Manganese toxicity has been demonstrated in nonhuman primates through intramuscular injections of manganese dioxide that lead to symptoms similar to Mn toxicity in humans (Pentschew *et al.*, 1963). The typical commercial diet containing approximately 70 to 100 mg Mn/ kg diet appears

adequate. In general, Mn toxicity through dietary intake occurs rarely in animals (Hurley and Keen, 1987) and only with concentrations of over 1000 μg/g diet. Dietary iron interacts with manganese and high levels of iron supplementation can lead to reduced stores of manganese (Hurley *et al.*, 1983).

f. ZINC. Zinc (Zn) activates enzymes needed in protein metabolism, is part of carbonate dehydratase, is prominent in carbon dioxide transfer, acts as a cofactor in a number of enzymatic processes, and is important for immune function. Zinc deprivation in pregnant rhesus monkeys from Days 110 to 150 of gestation resulted in rash, alopecia, anorexia, and low plasma zinc (Strobel *et al.*, 1977). Infants of Zn-deprived mothers were less active and displayed attachment behavior patterns which differed significantly from controls. The control diet contained 31 μg of Zn and produced no abnormalities (Strobel *et al.*, 1977; Sanstead *et al.*, 1978). A long series of studies of zinc deprivation in rhesus monkeys have comprehensively illustrated the effects of marginal deprivation of this mineral during pregnancy in the adult female monkey and on growth, development, and behavior of the offspring (see, for example, Swenerton and Hurley, 1980; Baly *et al.*, 1984; Golub *et al.*, 1984 a,b,c, 1985, 1988; Haynes *et al.*, 1985; Keen *et al.*, 1988; Leek *et al.*, 1984, 1988; Lönnerdal *et al.*, 1988). These studies provide excellent information on zinc in rhesus monkeys that cannot be detailed here. The control diet used for these studies contained 100 μg Zn per g diet. No cases of spontaneous Zn deficiency have been reported in nonhuman primates using commercial diets. The fact that zinc overload can lead to copper deficiency is well-documented and was discussed in the earlier section on copper.

Typical signs of zinc deficiency have also been produced in squirrel monkeys with a low zinc diet (Macapinlac *et al.*, 1967). Chadwick *et al.* (1979) reported a spontaneous zinc deficiency in marmosets (*Saguinus mystax*) that were fed a diet containing 150 μg Zn/g diet. The addition of Zn to the drinking water indicated improvement in signs of Zn deficiency with 40 ppm, but the abnormal fur coat returned with an addition of 80 ppm. The use of materials with known zinc contaminants (Smith *et al.*, 1985) in this early study raises questions about the actual levels of zinc in the plasma and diet. However, concern about species differences points to the need for additional studies of mineral requirements in all nonhuman primate species, particularly New World monkeys.

Commercial diets vary considerably in Zn content depending on other dietary components, however, 100 to 200 μg per g diet appears adequate. Rhesus monkey milk averaged 5.15 μg Zn/ml of milk during the first days of lactation and dropped to 1.86 μg/ml at 36 days postpartum (Lönnerdal *et al.*, 1984).

g. MOLYBDENUM. Molybdenum (Mb) is a constituent of the enzyme xanthine oxidase that plays an essential role in purine metabolism and is also a component of nitrate reductase. In animal nutrition, Mb is more widely recognized as a toxic element, particularly in combination with high levels of copper in herbage consumed by sheep (McDonald *et al.*, 1973). No studies of this element have been done in nonhuman primates and there is no recommended dietary level. Similar to cobalt, the trace amounts present in natural-based diets appear sufficient.

h. SELENIUM In conjunction with vitamin E, selenium protects biological membranes from oxidative degradation (Levander, 1986). Muth *et al.* (1971) reported that a dietary deficiency of Se in squirrel monkeys was reversed with a single injection of 0.04 mg sodium selenite. Lowe *et al.* (1975) described an accidental sodium selenite toxicity in *M. fascicularis* using a diet of 10.0 ppm Se instead of 0.1 ppm. The monkeys exhibited dermatosis on the tails, anorexia, leukopinia, and nail loss. The symptoms were reversed when the normal diet was resumed. Intramuscular injections of 0.22 mg sodium selenite plus 10.2 IU (±)-α-tocopherol/kg body weight proved more effective than either of the two nutrients independently in reversing hemolytic anemia in owl monkeys (*Aotus trivigatus*) in a study by Sehgal *et al.*, (1980). In a series of studies on Se metabolism with or without protein deficiency (Beilstein and Whanger, 1983; Beilstein *et al.*, 1984; Butler *et al.*, 1988), signs of deficiency were produced in rhesus monkeys using diets containing 15 or 30 ppb Se; the control was an addition of 200 ppb sodium selenite to the deficient diet. These authors only found cardiomyopathic lesions in the rhesus monkeys that received diets also deficient in protein. They proposed that the apparent resistence of rhesus monkeys to Se deficiency when compared with squirrel monkeys might be due to higher glutathione transferase activity in tissues of rhesus monkeys. Based on these studies, Se in the range of 0.01 to 0.02 mg/kg of diet appears adequate.

i. CHROMIUM. Chromium (Cr) deficiency results in impaired glucose tolerance, disturbances in lipid and protein metabolism, and impaired growth in animals. Supplementation of squirrel monkeys with trivalent Cr improved glucose tolerance in squirrel monkeys fed a commercial diet (Davidson *et al.*, 1967; Davidson and Blackwell, 1968). Although Cr deficiency is rare, the outcome in monkeys can be serious and diets providing approximately 150 μg Cr/day appear adequate (Martin *et al.*, 1972).

j. OTHER MINERALS OF NUTRITIONAL IMPORTANCE. Dietary requirements of the essential elements fluorine, tin, and other essential trace minerals have not been established for nonhuman primates. General estimates for nonhuman primates have been presented in the National Research Council report (NRC, 1978).

V. DIETS FOR CAPTIVE NONHUMAN PRIMATES AND SPECIAL CONSIDERATIONS

A. Natural Ingredient Diets

Several types of diets can be used to provide nonhuman primate colonies with their required nutrients. The most readily

available and frequently used diets are formulated with natural ingredients that are manufactured by extrusion. In this process the complete diet in meal form is subjected to high temperatures under steam pressure and is forced through a die resulting in a low density product that is highly palatable for most species of nonhuman primates. This type of nonhuman primate diet is readily available from commercial animal feed manufacturers. These commercial diets are formulated to meet the nutrient requirements of various nonhuman primate species. They are not formulated, however, to accommodate the requirements of research protocols requiring the use of diets with deficient or excessive nutrient concentrations. In order to meet such objectives it is necessary to formulate special diets. For instance, the formulation presented in Table II was for a diet with an increased crude fiber concentration that was originally used to determine the influence of dietary crude fiber on rhesus monkeys during quarantine (Morin *et al.*, 1978); it is presently used as a high fiber maintenance diet in various nonhuman primate colonies.

The nutrient stability of diets generally decreases as temperatures and humidity increase. The self life of any particular lot of feed is dependent on the environmental conditions of the storage area. Natural ingredient nonhuman primate diets stored in air-conditioned areas in which the temperature is maintained below 21°C (70°F) and the humidity below 60% should be used within 90 days of manufacture. This relatively short recommended shelf life for nonhuman primate diets as compared to 180 days for most other laboratory animal diets is due to the dietary supplement of vitamin C, the most labile nutrient required by these species. Nonhuman primate diets stored for longer periods of time, or under unusual environmental conditions, should be assayed prior to use for the labile nutrients (i.e., vitamin C, vitamin A, and thiamin) to ensure adequate concentrations. Diets formulated without antioxidants or with large amounts of highly perishable ingredients such as fat might require special handling or storage procedures. There are two concerns about the use of natural ingredient nonhuman primate diets, particularly in colonies involved in biomedical research where diet is a potential variable significantly influencing experimental results. Factors such as varieties of plants, soil conditions, weather conditions, harvesting or storage procedures, and manufacturing or milling methods influence the nutrient composition of natural ingredients to the extent that production batches of a diet are not identical in nutrient composition (Knapka, 1983). This variation in dietary nutrient concentrations introduces an unknown variable that can have a significant influence on certain experimental results. Natural ingredients can also be subjected to various naturally occurring or manmade contaminants such as pesticide residues, heavy metals, estrogen activity, and numerous other compounds. Diet manufactured from these ingredients can contain low concentrations of contaminants that generally have no influence on animal health, but may alter experimental results (Rao and Knapka, 1987).

TABLE II

FORMULATION OF NIH OPEN-FORMULA, HIGH-FIBER NONHUMAN PRIMATE DIET

Ingredient[a]	Amount
Diet formulation[b]	
Ground wheat (10% protein)	280
Alfalfa meal (17% protein)	30
Ground oat by-product	225
Soybean meal (48% protein)	80
Fish meal (70% protein)	60
Brewer's dried yeast	20
Dried whey product	30
Ground corn	170
Sugar	29
Soybean oil	36
Salt	3
Dicalcium phosphate	5
Calcium carbonate	12
Mineral premix	10
Vitamin premix	10
	1000
Mineral premix[c]	
Cobalt	0.44
Copper	2.20
Iron	33.04
Magnesium	440.53
Manganese	15.42
Iodine	1.43
Potassium	991.00
Zinc	4.41
Extender to	10,000.00
Vitamin premix[d]	
Vitamin A	10,682.82 IU
Vitamin D_3	5,947.14 IU
Vitamin E	40.74 IU
Vitamin B_{12}	6.60 μg
Riboflavin	5.29
Niacin	57.27
Pantothenate	55.07
Choline	323.79
Menadione Activity	8.80
Folic acid	2.64
Pyridoxine	8.80
Thiamine	19.82
Biotin	0.11
Vitamin C	1.10
Extender to	10,000.00

[a]Amount per kilogram of diet.
[b]Values in grams.
[c]Values in milligrams.
[d]Values in milligrams unless noted.

B. Purified and Liquid Diets

Purified diets have been formulated for Old World and New World species of nonhuman primates (NRC, 1978). These diets are formulated with ingredients that have been refined to the

degree that each one essentially contributes a single nutrient or nutrient class to the diet allowing the production of multiple batches of diet with minimal variation in nutrient concentration. Purified diets have not been extensively used for nonhuman primates because they are of low palatability to most species and they are considerably more expensive than the more readily available natural ingredient diets. However, the use of purified diets may be required for studies where variations in dietary nutrient concentrations potentially influence experimental data. For instance, data from studies designed to establish nutrient requirements or to study immune function are subject to influence by variations in dietary nutrient concentrations.

Liquid diets also have been formulated for nonhuman primates (NRC, 1978) and are primarily used for the care of newborn animals. They may also have application in studies involving alcohol or other test substances in liquid form.

C. Canned Diets

Canned diets containing high-moisture concentrations are commercially available for nonhuman primates and are frequently used for the smaller species. All canned diets may not be formulated as a complete diet for all species of nonhuman primates. A direct comparison of the nutrient composition data provided on labels with estimated nutrient requirements or the composition of dry diets is not valid since it is necessary to first convert the contents of the canned diets to a dry matter basis. Canned diets are highly palatable and are generally readily consumed by nonhuman primates; however, based on the moisture content they are considerably more expensive than dry diets.

D. Pelleted and Baked Diets

Diets for nonhuman primates are also manufactured by pelleting and baking. Generally the high density pelleted diets are not as palatable for nonhuman primate species as the low density extruded diets. However, the maximum amount of fat that can be provided in extruded diets is 5 to 6% of the total diet because of technical limitations of the extruding process. In order to provide diets for species with higher energy requirements, it is necessary to provide diets in a different form. For example, Barnard *et al.* (1988) formulated a diet for marmosets and tamarins containing a minimum of 10% crude fat that was pelleted. The addition of dietary fat at this concentration resulted in relatively soft pellets that were readily consumed by these species.

The manufacture of nonhuman primate diets by baking is an option used to provide diets for breeding colonies that have been established in developing countries near the natural habitat of the animals. It is generally impractical to ship manufactured diets into these areas and conventional feed manufacturing facilities are not available. Diets can be formulated with locally available ingredients, but they must be subjected to a process involving heat such as baking to decrease pathogenic organisms and to gelatinize starches in order to avoid the gastrointestinal problems that frequently occur when nonhuman primates consume raw starches.

TABLE III

NUTRIENT CONCENTRATIONS OF LIVE FOOD OFTEN FED TO NONHUMAN PRIMATES

Nutrient	Cricket	Mealworm	Waxworm	Newborn mouse
Moisture (%)	71.0	66.4	56.1	81.4
Crude protein (%)	19.4	18.3	13.5	12.2
Crude fat (%)	4.9	11.0	27.0	4.3
Crude fiber (%)	2.8	2.2	0.9	0.2
Ash (%)	2.4	2.3	0.8	1.8
Calcium (%)	0.03	0.01	0.01	0.30
Phosphorus (%)	0.23	0.19	0.17	0.33
Sodium (%)	0.11	0.03	0.02	0.16
Potassium (%)	0.32	0.29	0.23	0.31
Magnesium (%)	0.04	0.06	0.02	0.02
Cobalt (mg/kg)	Trace	0.04	0.05	0.05
Copper (mg/kg)	6.2	5.4	2.6	3.2
Iron (mg/kg)	22.2	15.0	19.4	47.5
Manganese (mg/kg)	7.7	2.7	1.1	0.8
Selenium (mg/kg)	2.1	0.2	0.3	0.2
Zinc (mg/kg)	53.9	64.8	19.0	16.1
Carbohydrate[a]	—	—	1.2	0.2

[a]Nitrogen-free extract.

E. Succulent and Live Foodstuffs

Various succulent foodstuffs such as fruits, vegetables, and other products are frequently fed to nonhuman primate colonies as feed supplements. These highly palatable, readily consumed foodstuffs add variety to the diets of captured nonhuman primates. However, a large percentage of these foodstuffs contain high concentrations of moisture and low concentrations of energy or protein as compared to diets that are formulated for nonhuman primates. There is a concern that when succulent feedstuffs are fed *ad libitum* they become a substitute for more nutritious food because given the opportunity nonhuman primates tend to consume the more palatable rather than the most nutritious foodstuff. The amount of succulent foodstuffs offered to nonhuman primates should always be controlled to ensure that the total diet they consume provides adequate amounts of the required nutrients.

The practice of providing live food for captive nonhuman primates may satisfy a psychological requirement, but it does not provide significant amounts of the required nutrients. The data presented in Table III indicate that moisture is the most abundant nutrient found in live foods frequently fed to nonhuman

primates. Based on these data the waxworm would appear to be the most nutritious insect because of its crude fat content. However, on an actual weight basis, large numbers of these insects would have to be consumed to provide a significant amount of energy.

F. Dietary Supplementation as Environmental Enrichment

One important auxiliary function of the nonhuman primate diet is its use for providing positive behavioral stimulation as a means of enhancing the primate's well-being (Federal Register, 1991). New federal regulations stipulating providing "environmental enhancement" to laboratory primates can be met in a variety of ways [Bayne, 1989; Fajzi *et al.*, 1989; National Institutes of Health (NIH), 1991]. In general, these various methodologies are used to evoke species-appropriate behavior in the captive animal. Activities that can be fostered in the laboratory setting include components of social, manipulative, locomotive/postural, and foraging behavior.

Using food supplements or treats to provide environmental enrichment has additional positive value because foraging constitutes a major part of the time budget for free-ranging primates. Foraging related activities have been shown to occupy 7–65% of the diurnal activity budget of free-ranging monkeys (Milton, 1980; Herbers, 1981; Strier, 1987; Malik and Southwick, 1988; Marriott, 1988; O'Neill *et al.*, 1989), and have been described as "the single most time-consuming behavior" during some seasons of the year (Malik and Southwick, 1988).

The opportunity for primates to engage in foraging activities in the laboratory has only recently been made available. Traditionally, laboratory primates were fed a nutritionally balanced meal once or twice a day. Caged monkeys typically consume a meal in an average of 47 min (Bayne *et al.*, 1991), representing only 6.5% of the 12-hr light cycle per meal. Increasing the amount of time the laboratory primate spends in eating behaviors can be accomplished in a variety of ways using nutritionally complete food treats now commercially available. These commercially available food treats come in a variety of sizes, colors, shapes, and flavors. This diversity allows for tremendous flexibility in designing an enrichment program centered on foraging behavior.

Five general methods of food delivery are used to increase the time spent eating: (1) the food is scattered over a large surface area so the animal is required to move through more of its environment to obtain food; (2) the food is placed in a device or substrate in such a way that the animal must search for it; (3) the food item is reduced in size (e.g., made particulate) so more time is spent in retrieving and consuming a small volume of food; (4) the animal must perform a task to obtain food that is visually available; or (5) the food requires processing (e.g., removing a shell or husk). Several foraging devices are available commercially and many are "in-house" fabrications. There is a substantial range of price, ease of integration with husbandry procedures, sanitizability, and durability with these products.

Devices designed to stimulate foraging behavior include puzzle feeders (Line and Houghton, 1987), time-consuming devices such as foraging boards (Moazed and Wolff, 1988; Bayne *et al.*, 1991), PVC knots in which the food must be massaged down a clear flexible tube to be retrieved (Bayne, 1989), gum-gouging containers for some New World species (McGrew *et al.*, 1986), food-dipping devices for chimpanzees which mimic behaviors observed in free-ranging chimpanzees (Maki *et al.*, 1989), fingerboards for chimpanzees (Brent, 1991), and devices which require searching for the food that are mounted on the cage wall or suspended in the primary enclosure (Rosenblum and Smiley, 1984; Beckley and Novak, 1989; Hayes, 1990). In some cases, food treats can be delivered to the primates by dropping the food into a substrate that partially obscures the food item from view. This has been successfully applied to groups of animals housed in primary enclosures with wood shavings on the floor (Westergaard and Fragaszy, 1985) and to primates living in cages that have wood wool on the floor (NIH, 1991). Additionally, food can be delivered to the primates by hand. This technique, although very simple, encourages positive interaction between the animals and the care givers. Because of the potential increased safety risk to the personnel involved in this task, training of the staff and selective application of this method only to animals that meet certain behavioral criteria (e.g., animals which are not overly aggressive) are essential.

Because of the hierarchical nature of some primate societies, and the resulting competition over scarce resources, caution must be exercised when providing a social group with nutritive environmental enrichment. High-ranking animals may hoard supplementary food treats intended for the group by monopolizing an enrichment device. Clearly, this prohibits the other group members from benefiting from the enrichment and can occasionally result in increased intragroup aggression. Also, an animal that tends to dominate a food source is at a heightened risk of bloat (gastric dilatation). To prevent these problems, multiple foraging devices can be dispersed around the primary enclosure. Similarly, if food is hidden in a substrate on the floor of the enclosure, it should be spread evenly on the floor instead of piled up in a small area.

Dietary supplementation can be an economic method of enhancing the environment of laboratory primates. This class of enrichment can evoke the expression of greater amounts and diversity of normal foraging behaviors. With the advent of commercially available nutritionally balanced food supplements, the days of treating nonhuman primates with candies and marshmallows should be past.

G. Special Consideration: Wasting Marmoset Syndrome

Marmosets and tamarins, members of the family Callitrichidae, have great potential as laboratory animal models for human

diseases (Epple and Katz, 1983; Clapp *et al.*, 1985; Johnson, 1985). However, progress in the use of callitrichids in research has been impeded by the occurrence of what has been termed wasting marmoset syndrome. The etiology of WMS has not been determined, but it is believed to be due to behavior, infection, malabsorption, and/or malnutrition. There are six characteristics associated with WMS: weight loss, muscle atrophy, hair loss, chronic diarrhea, chronic colitis, and anemia (Morin, 1983).

Chadwick *et al.* (1979) noticed that some of these characteristics are similar to the clinical signs of zinc deficiency. They reported a dramatic improvement in the alopecia and dermatitis of the wasting monkeys upon adding 40 ppm zinc to the drinking water. However, zinc deficiency can be secondary to protein deficiency. Barnard *et al.* (1988) showed that wasting *S. mystax* with low serum zinc concentrations were protein–calorie deficient, and that these conditions could be reversed by dietary intervention. Shimwell *et al.* (1979), Kirkwood *et al.* (1983), and Tucker (1984) have suggested that WMS may be related to protein deficiency.

Wasting marmoset syndrome may also be related to the feeding behavior of callitrichid family groups according to Tardif and Richter (1981). They observed that in callitrichid family groups social interactions result in inequalities in food consumption among group members which could result in some monkeys receiving an inadequate diet.

Shimwell *et al.* (1979) and Barnard *et al.* (1988) have cautioned the scientific community that dietary regimens that provide excessive supplementation could result in WMS. Evidence exists that excessive dietary supplementation results in the callitrichids eating preferred foods, such as fruits, and neglecting the more nutritious foods (Shimwell *et al.* 1979; Kirkwood *et al.*, 1983; Barnard *et al.*, 1988). Marmosets in captivity thus warrant careful dietary and behavioral monitoring to avoid animal deaths due to WMS.

VI. CONCLUSION

It is well known that the nutritional status of animals used as models in biomedical research has a profound influence on the quality of data obtained during experimental protocols. In addition, adequate nutrition affects the well-being and life quality of captive nonhuman primates. The amount of knowledge regarding the dietary and quantitative nutrient requirements of nonhuman primate species is inconsequential compared to other species of laboratory animals, even though various species of nonhuman primates have been used in biomedical research for many decades. In order to improve the value of nonhuman primates as animal models and to contribute to their well-being it is essential to conduct research designed to determine the quantitative nutrient requirements of the specific nonhuman primate species. Quantitative nutrient requirement data on nonhuman primates are needed if we consider designing new diets that could incorporate information that caloric restriction may be advantageous to the well-being of laboratory animals.

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CHAPTER 12

Animal Identification and Record Keeping: Current Practice and Use

Bennett Dyke

I. INTRODUCTION

The task of record keeping and tracking the whereabouts, use, health, and reproductive status of animals is an important one in most nonhuman primate colonies. Most managers have found that managing colony information has grown to be a major part of their operations and that investment in computer hardware, software, and personnel has expanded accordingly. This situation has come about gradually as the value of breeding animals has grown, the availability of relatively inexpensive imports has declined, and administrative burdens and expenses have increased with the emergence of ever more intricate regulatory demands. The larger institutions have had a longer experience with these issues, but ever more complex record keeping is now a ubiquitous colony feature no matter what the size of the population of animals.

II. ANIMAL IDENTIFICATION

A. Identification of Individuals

Fundamental to keeping records is the unambiguous permanent identification of individual animals. Unfortunately, no really satisfactory identification method has been devised to date. Tattooing a number or code is probably the most common method, but has a major drawback in that it usually requires the animal to be immobilized for reading. Tattoos also require

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periodic renewal because cutaneous inks tend to diffuse over time, particularly in growing tissue. Collars and identification tags make it possible to identify free-ranging individuals and are used with some New World species and with baboons. Nonetheless, tags are best used in conjunction with tattooing since even the most rugged attached materials tend to be lost or destroyed, which may leave the animal otherwise unidentified. Dye marking is particularly useful for observations of free-ranging animals at relatively long distances, but requires systematic renewal as hair is shed. Freeze branding has been tried occasionally (Hadow, 1972), but the technique is unsatisfactory except in short-haired animals. Small radio frequency microresponders have gained increased use. These "chips," small sealed cylinders about 1.5 mm in diameter and 100 mm long, are permanently implanted subcutaneously. They require no internal battery power, but in a sense "reflect" a coded digital signal when activated by a radio transmitter. A drawback is that the transceiver device used to activate and read the signal from the implant operates only within a range of a few inches, which means that animals must be physically positioned (if not restrained) for reading. Another problem lies in the fact that coding has not yet been standardized so that implants supplied by one manufacturer cannot be read by equipment provided by another. However, the industry may set standards, particularly now that the domestic animal and pet markets are being targeted. A more serious impediment to widespread adoption at the present time is the cost of the implants and equipment. Nonetheless, when prices decline sufficiently, implantables will most likely become the identification method of choice, even if the reading range cannot be improved significantly.

B. Identification of Pedigree Relationships

Another important dimension exists in animal identification in addition to simply making sure that one individual is not mistaken for another. This is identifying the individual with respect to the relationships that connect it to members of its family; that is, identifying its pedigree relationships. In effect, what is required is an unambiguous identification of the paternity and maternity of each animal in a breeding population. In some colonies it is possible to approach this goal by caging breeding age animals either individually or in pairs so that sire and dam are known. However, it is apparent that even under these circumstances that a certain proportion of parents (on the order of 5–10%) are misidentified because of recording and handling errors, escapes, intercage copulations, maternal theft, and switching of infants. A more satisfactory method of establishing paternity and verifying maternity is to use genetic marker typing. The major drawback of marker typing is that it is time-consuming and expensive, but it has the advantage of reducing pedigree errors to extremely low levels. Once identity has been established, the ability to cross-check vital statistics and other management information between family members offers an extremely powerful addition to data validation procedures. Pedigrees are also crucial for genetic analyses, which depend on detecting Mendelian transmission patterns underlying the distribution of phenotypes in families. These methods are certain to become a more important aspect of disease model development as the human gene map fills out and as serious efforts at mapping begin in nonhuman primates. Even when study goals are not explicitly genetic, pedigrees can greatly enhance the value of research animals—the assumption of independence between experimental subjects is a risky one if the animals are all derived from the same interbreeding colony. Likewise, many demographic analyses (particularly fertility measures) require knowledge of parent–offspring identity.

III. RECORD KEEPING

In 1979 a Committee on Laboratory Animal Records was established by the National Research Council in recognition of the need for common data management practices for effective primate colony management. In its report (National Research Council, 1979), the committee specified criteria for a record keeping system that would (a) identify basic information needed for local colony management, (b) provide basic data for evaluating breeding programs, (c) provide a uniform compilation of information that can accompany individual animals that are transferred between colonies, and (d) define a set of uniform record items so that information can be shared among institutions and used for national planning.

Extending this work, an *ad hoc* committee formed at a workshop held as part of the program of the 1987 meeting of the American Society of Primatologists (Dyke *et al.*, 1993) specified a set of guidelines for standardizing primate colony data. Variables included in the standard were limited to the minimum required for basic demographic analysis and for the construction of extended pedigrees for population genetic analysis. In addition, the directors of the U. S. Regional Primate Research Centers at their annual 1990 meeting assembled a minimal set of reproductivity and mortality measures to be used for reporting purposes and to evaluate reproductive efficiency in nonexperimental, group-housed monkey populations (Hendrickx, 1990).

Because of the high cost of early technology, computerized record keeping started with the larger institutions that could afford to develop their own data management software, which was usually specific for a particular brand of large central computer. Storage capacities were limited, which necessitated complex data coding that represented the largest amount of information in the most compact form possible. All aspects of data management were by necessity done by highly specialized technical personnel. Data retrieval frequently required knowledge of programming techniques, decisions about additions and changes to databases and modifications of data codes were often

major considerations, and a changeover to a new computer (even a new model of the same brand) was likely to be a seriously disruptive process.

Today, however, small desktop computers are powerful enough to manage large databases using inexpensive database software that incorporates the same sophisticated data manipulation techniques found on large mainframe applications. Because these packages have been implemented for a broad, nontechnical clientele, they have been made considerably easier to use than much of the older software. The dramatic decline in the cost of data storage has also reduced the need for cryptic data coding, meaning that information can be stored in a form that is interpretable to the eye. With many of the technical barriers removed, data management may now be in the hands of those who know the data best, and data requirements *per se* can be the sole determinant of issues in colony record keeping. These advances make it possible for even the smallest colonies to enjoy the advantages of computer management of their data.

TABLE I

STANDARD SINGLE-ENTRY REGISTRY RECORD STRUCTURE

Variable	Mnemonic	Length	Type	Description
1	EGO	3–10	Character	Animal ID
2	SIRE	3–10	Character	Sire ID
3	DAM	3–10	Character	Dam ID
4	SEX	1	Character	Sex
5	BIRTH	6–15	Date	Birth date
6	ENTRY	6–15	Date	Entry date
7	AQCODE	2–6	Integer	Acquisition code
8	EXIT	6–15	Date	Exit date
9	EXCODE	2–6	Integer	Exit code
10	TAXON	3	Character	Taxonomic code
11	INSTT	6	Character	Institution code
12	SUBGRP	3–10	Character	Local subgroup code
13	LOC	3–10	Character	Current location code
14	EOR	1	Character	End-of-record character

IV. DATA STANDARDIZATION

Animal colony data fall into two classes, each of which requires a somewhat different approach to record keeping and analysis. *Single-entry data* apply to events or measures that arise only once during the lifetime of the individual. Individual vital statistics, including IDs of the individual and its parents, sex, dates of its birth, and death, are kept in the *registry* (following human vital statistics terminology). Registry data are usually stored separately from other single-entry data, such as necropsy records and genetic markers. *Multiple-entry data*, on the other hand, are those which derive from events or measures that may be repeated throughout the lifetime of the individual animal. These include information that comprise clinical, reproductive, developmental, and experimental histories.

A. Standardized Single-Entry Registry Record Structure

Variables included in a primate colony registry are often limited to those required for demographic analysis, such as computation of birth and death rates, and for construction of extended family trees (pedigrees) used in genetic analysis. A set of these variables is kept on a single computer file record for each individual that has ever been resident in the colony. Two ways to configure a simple computer database are (a) to specify a fixed field length for each variable, making records for all individuals identical in length, or (b) allowing records to vary in length, keeping fields separated by special characters such as tabs or commas. No matter which structure is used, it is best to avoid coding the variables with complex schemes that are difficult to remember or interpret by eye. Table I lists 14 variables selected by the ad hoc standards committee as fundamental to records in a colony registry. The list includes format information for fixed-length computer records, although variable-length records are often used. This structure was based on the conventions of the International Species Inventory System (ISIS) Animal Records Keeping System (ARKS) database (Scobie, 1987; Seal *et al.*, 1977), with modifications derived from a survey of databases from several major colonies. The rationale for this choice of variables is as follows.

Variables 1–3. The presence of SIRE and DAM IDs on EGO's record makes it possible to identify sibships and to compute aggregate summaries of fertility by sire and dam. EGO-SIRE-DAM triplets also are the building blocks for construction of extended pedigrees.

Variables 5–7. A distinction is made between date of birth and date of first entry into the colony population. This is because age is computed from birth date, whereas duration of residence in a colony (an important consideration for demographic analysis) is computed from date of entry. Acquisition codes specifying the origin of the animal may be useful in computing statistics for subsets of colony members (colony-born, wild-born, etc.).

Variables 8 and 9. In contrast to birth date, a single exit date combined with standardized exit codes is sufficient for the registry standard. This is because deaths of animals are not tabulated if they have previously left the colony for some other cause (sale, trade, etc.). No matter how many exit codes are used, it must be possible to group them into four analytically relevant classes (alive and resident at time of census or count, natural death, exit while alive, or unknown cause of exit).

Variables 10–14. Taxonomic, institution, local subgroup, and current location codes help in identifying the population and colony, but are usually not necessary for demographic or genetic analysis. An end-of-record character is useful in assuring that formatted records are the same length.

Other single-entry data not related to vital statistics are usually kept in their own computer files. For example a *birth weight*

TABLE II

SINGLE-DATE RECORD STRUCTURE

Variable	Mnemonic	Length	Type	Description
1	EGO	3–10	Character	Animal ID
2	CHANGE	6–15	Date	Status change date
3	STATUS	1–6	Character	Status code
4	EOR	1	Character	End-of-record character

file might be made up of records containing only an animal ID and a measure of weight, whereas a *marker file* would contain only genotype information.

B. Standardized Multiple-Entry Records

Collecting information from recurring events can present data storage problems, even for computers. The *ad hoc* standards committee defined two frequently used multiple-entry record structures based on the assumption that it often makes more sense to keep a separate record for each event instead of allowing for individual records to grow to unwieldy proportions as data for them accumulate. The *single-date format* assumes that an animal starts out in a certain state (for example, in a single-sex gang cage), and a new record is entered for that individual each time that state changes subsequently (for example, each time the animal is moved in or out of a breeding cage). These records contain the animal ID, the date of the change of the status, and a code defining the nature of the status. The standardized record structure is shown in Table II.

The principal advantage of this format is that only the beginning of an event or status change is recorded so that events may be entered as soon as they occur without regard to their end, which is marked by the start date of the next event, etc.

In contrast, the *two-date format* keeps both the start and stop dates of the event on the same record, as shown in Table III. This structure is more convenient for sporadic events of shorter duration (such as hospitalization or clincial treatment) and has the advantage that event histories are self-contained on a single record.

TABLE III

TWO-DATE RECORD STRUCTURE

Variable	Mnemonic	Length	Character	Description
1	EGO	3–10	Character	Animal ID
2	START	6–15	Date	Status change start date
3	STOP	6–15	Date	Status change end date
4	STATUS	1–6	Character	Status code
5	EOR	1	Character	End-of-record character

Multiple-entry records are usually kept in separate files that define particular kinds of events or states; for example, a *location file* would contain only records defining cage changes, whereas a *fertility file* might consist of reproductive timing data for individual females. According to this scheme, complex individual histories can be assembled from records identified by animal ID distributed *within* a file. Likewise, the animal ID links information for a given individual *across* both single- and multiple-entry files. Database software that depends on this so-called *relational* structure is commonly used in animal colony data management. Further detail on record structure and coding can be found in Dyke *et al.* (1993).

V. REPORTS AND ANALYSES

The ultimate reason for identification and record keeping is, of course, the need for monitoring the health, well-being, and productivity of the colony. Colony status reports are fundamental to any well-run colony, and for the most part can be generated from the colony vital statistics registry, plus any number of additional multiple-entry data files.

A. Day-to-Day Management Reports

Management reports tend to be tailored to the needs of individual colonies. Typically, these are produced both periodically (that is, daily, weekly, monthly, and yearly) and on demand, and include formatted summaries of information from the registry and all other data files. Contents include vital statistics, project histories, experimental schedules, clinical and surgical case histories, reproductive histories, morphometrics, and animal-related grant and financial accounts.

B. Standard Demographic Measures

Published sources on demographic methods for nonhuman primate populations are for the most part based on the requirements of ecological research (for example, National Research Council, 1981), where estimating parameters from incomplete field data is a major concern. With suitable modifications, human population methods are more appropriate for captive colonies that have maintained adequate registry information. The study of the demography of human populations has been successful for two reasons. First, extensive experience with a wide variety of human data facilitates standardized comparisons among populations and a meaningful interpretation of analyses. Second, a long history of research has produced highly developed methodology and techniques for measurement and analysis (see Barclay, 1958, and Pollard *et al.*, 1981, for excellent elementary expositions of these methods). Although elaborate demographic analyses are not necessary for day-to-day opera-

tions, they often reveal patterns of mortality, fertility, and growth that otherwise may go undetected.

A sensitive measure of colony mortality is the epidemiologic life table, which is designed to take into account (a) the likelihood that individuals are of differing ages at the beginning of the registry period, (b) the possibility that individuals may join the population at some time after the beginning of the registry period, (c) the possibility that individuals leave the population before the end of the registry period for reasons other than death (in epidemiological terms, *lost to follow-up*), and (d) the virtual certainty that individuals present in the population at the end of the registry period (termed *withdrawn alive*) will be of differing ages. Although not initially intended for population analysis, it is clear that epidemiologic methods are ideally suited for the construction of life tables for nonhuman primate colonies. The general strategy for analysis of these populations is to give a demographic (as opposed to clinical) interpretation to epidemiologic life table measures. An additional advantage of the epidemiologic life table is that it permits estimates of standard errors of its entries. This, like most mortality measures, can be derived from single-entry records in the registry file.

Some conventional measures of human fertility are also useful as standards against which to assess the reproductive performance of individual animals. For example, the *total fertility rate* is a population measure of births to females known to be at risk of reproducing, that is, tabulations of births are made only during the precise intervals if time that a female is receptive and is caged with a breeding age male. This measure discounts reproductive downtime and represents an ideal fertility performance for the colony which can be compared to the *completed family size*. The latter measure is simply the sum of live births actually produced by an individual female over her reproductive lifetime. The difference between these measures indicates the effects of downtime (for example, when the individual is not in a breeding cage) or the existence of some other impediment to full reproductive potential. Most measures of fertility depend on multiple-entry data recording and on pedigree identification of individuals.

Appropriate aggregate measures of fertility and mortality make it possible to predict the size and age–sex structure of the colony a few years into the future. *Projection models* of this sort are particularly useful if they are combined with a financial component so that estimates of future cost can be made.

Standard demographic techniques deserve wider utilization as primate colony management tools, but the software technology required is surprisingly complex and has not been extensively implemented (see Dyke, 1989b, and Scobie, 1987, for two examples).

C. Monitoring Genetic Management

An increasingly important colony data management concern comes with the recognition of the need for maintaining genetic variability in the population and for producing animals for research whose family relationships are known. Indentification of animals with respect to pedigree is of course critical here. Specialized computer programs may be useful for determining relationships when genetic markers are used to establish paternity, but the basic management task is to structure matings so that the contribution of original colony founders to ancestry of living animals is equalized as much as possible and so that close inbreeding is avoided. Colony management software should include these functions (Dyke, 1989a; Scobie, 1987).

VI. SUMMARY

The unique identification of animals, including the identification of family relationships, is a critical necessity in colony management practice and is closely linked to methods of record keeping. Inexpensive hardware and database software have made computerized data management a nearly universal practice, which has led to an increasing standardization of data content and format. Reporting and analysis are easily programmed at the local colony level to fit individual needs, although specialized software is required for demographic and genetic analysis.

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CHAPTER 13

Medical Management

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NONHUMAN PRIMATES IN BIOMEDICAL RESEARCH: BIOLOGY AND MANAGEMENT

PART A. PREVENTIVE MEDICINE

Janice L. Southers and Elizabeth W. Ford

I. INTRODUCTION

Nonhuman primates are a valuable and limited research resource closely related phylogenetically to humans. The challenge to the laboratory animal veterinarian responsible for nonhuman primates is to provide a quality research animal, to protect personnel from zoonoses, and to protect valuable established colonies of nonhuman primates. A comprehensive preventive medicine program can accomplish these goals and reduce the cost associated with disease outbreaks and medical care (Jonas, 1976; Kaufmann, 1971).

Although preventive medicine programs may vary between species and specific situations, some general concepts always

apply. The foundation of any healthy colony begins with a comprehensive quarantine with diligent professional oversight. Other crucial elements include quality husbandry, health surveillance, and programs for disease prophylaxis.

II. QUARANTINE

A. Goals of Quarantine

The basic goals of quarantine are to protect the existing colony from the introduction of infectious diseases, to protect personnel from the introduction of zoonotic agents, to minimize the transmission of diseases between animals being quarantined, and to optimize the health and condition of newly acquired animals. These goals are accomplished by maintaining strict separation between quarantine facilities and the rest of the colony, maintaining small stable groups during quarantine, and examining and screening animals for diseases. Quarantine groups should be established by species, disease status (where known), and source and date of arrival in quarantine. Other factors to consider when establishing quarantine groups are the age of animals and existing social history. The length of quarantine and the extent of examinations and screening procedures are determined by the source and condition of the animals, the value of the colony at risk, and, for primary importers, federal regulations.

Unlike many other laboratory species, nonhuman primates often come from a wide variety of domestic and nondomestic sources. The disease status varies with the source and level of conditioning. Their flora is generally not as well defined as other laboratory species and they represent a much greater zoonotic potential.

B. Source of Animals

In general, the highest quality nonhuman primates are available from domestic (United States) sources. These animals have been bred or held in this country for a number of years, and therefore have a defined level of conditioning. Currently the Public Health Service sponsors programs developing a stable defined group of specific pathogen-free animals that are bred and maintained in this country [National Center for Research Resources (NCRR), 1991]. When these colonies are established they will represent a valuable animal resource, free of specified diseases, but will require separation from conventional animals. Another source of quality animals are those which are captive bred and conditioned in their native country and exported for research. Animals that are wild caught and held for a variable amount of time before export to this country have a greater risk of disease. Importing animals with no conditioning poses the greatest risk of disease. Although wild-caught or unconditioned animals have the greatest potential for illness and disease, even animals from reliable domestic sources should be rigorously quarantined.

C. Quarantine Regulations

Federal requirements for primary quarantine facilities state that any facility which holds, receives, or imports nonhuman primates must abide by all local, state, and federal regulations. Since October 10, 1975, the importation of nonhuman primates has been restricted by Public Health Service quarantine regulations. Importation of live nonhuman primates is permitted only for bona fide scientific, educational, or exhibition purposes, by importers registered with the Centers for Disease Control (CDC). Nonhuman primates may not be imported into the United States as pets [Code of Federal Regulations (CFR), 1975].

No person or organization may import live nonhuman primates into the United States unless registered as a primary quarantine facility with the CDC. The requirements for quarantine are more stringent for facilities which import directly from source countries or receive animals within 31 days of importation into the United States. These sites are referred to as primary quarantine facilities. Secondary quarantine facilities only receive animals previously conditioned 31 days at a primary quarantine facility. (CFR, 1987).

Appropriate records on each shipment must be maintained in accordance with requirements published in the Code of Federal Regulations, Title 42. In addition, the importer must obtain signed and dated documents from the recipient of any imported nonhuman primates to establish that the recipient will use these primates solely for the permitted purposes.

Importers must report by telephone, within 24 hr of occurrence (including nights, weekends, and holidays): (1) any illness in nonhuman primates that is suspected of being yellow fever, monkeypox, or Marburg/Ebola disease, and (2) illness in any member of their staff suspected of having an infectious disease acquired from nonhuman primates (CFR, 1975).

The director of the CDC is authorized upon receipt of evidence of exposure of nonhuman primates to a communicable disease that constitutes, or may constitute, a threat to public health to provide for or require examination, treatment, detention, quarantine, seizure, or destruction of exposed animals.

Import and export of many nonhuman primates are regulated by the U.S. Department of the Interior, Fish and Wildlife Service under the Endangered Species Act (ESA) (CFR, 1973) and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (1977).

All nonhuman primates must enter and leave this country through customs ports designated by the U.S. Fish and Wildlife Service. An Exception to Designated Port permit can be obtained from the U.S. Fish and Wildlife Service, Division of Law Enforcement under special circumstances. A "Declaration for Importation or Exportation of Fish and Wildlife" form must be

filed upon import or export [U.S. Department of the Interior (USDI), 1985].

All nonhuman primates must be transported in accordance with the Animal Welfare Act (AWA) (CFR, 1985). In addition, it is the responsibility of the facility veterinarian to be cognizant of all state, city, and county regulations which may be more stringent than federal regulations.

D. Quarantine Facilities

Ideally the quarantine facility should be located at a different location than the colony and it should not share equipment, clothing, personnel, or transport vehicles. This serves to minimize the probability of cross contamination. If a separate location is not available, the quarantine area, room, or building should be located on the periphery of the core facility and no sharing of caging, equipment, or, ideally, personnel should be permitted.

A dual-corridor floor plan leading to a three-room changing area (soiled locker room, shower pass through, and clean locker room) is ideal for a quarantine facility. If this is not possible, each room should have an attached anteroom with a counter and sink and the facility should have a central locker room [Department of Health and Human Services (DHHS), 1988]. The anteroom serves two purposes: it places two doors between the animals and the rest of the facility, providing extra security and it provides a place to examine and treat animals. Several small rooms are preferable to one or two large rooms. This allows the acceptance of sequential shipments of animals without extending the quarantine period for earlier shipments and will help contain an infectious disease outbreak to smaller numbers of animals. Additional design considerations are sweeps and rubber seals for the doors, an autoclave in the soiled corridor or in very close proximity for sterilizing contaminated materials, exhausted air should not be discharged in the vicinity of the air intake, and traps on the floor drains should contain and disinfect waste prior to disposing into the sewer system. All other specifications of the quarantine facility are the same as for animal holding rooms as described in "*The Guide for the Care and Use of Laboratory Animals*" (DHHS, 1985).

E. Infection Control Procedures

Animal Biosafety Level 2 facilities and procedures should be adequate for most quarantine groups. If an outbreak of potentially zoonotic disease occurs, Animal Biosafety Level 3 practices should be initiated and the proper authorities notified as required (DHHS, 1988).

Only authorized trained individuals should be allowed access to quarantine. Ideally these individuals should only work with animals in quarantine. If they must work in the established colony, this should be done prior to entering quarantine. Protective clothing should include a long-sleeved isolation gown, a dust/mist mask, mucus membrane protection (e.g., goggles or face shield), gloves, and separate shoes or shoe covers. The protective clothing should be disposable or reusable clothing should be autoclaved prior to being laundered.

Animals should not be handled unless chemically immobilized. An exception to this is the receipt of the animal when the animal can be carefully transferred from a non-squeeze transport container to a cage without immobilization.

Individuals working in the quarantine area should be included in a comprehensive occupational health program as described in Chapter 15.

F. Duration of Quarantine

The length of time for quarantine should be determined by the value of the animals in the colony to be protected. A large breeding colony or a group of animals that have undergone technically difficult surgeries or have been on experimental studies for several years is a very valuable resource and should be protected carefully. Although the legal federal minimum is 31 days (CFR, 1987), a more suitable quarantine period is 90 days. This allows time for adequate detection of underlying disease. Animals that are immunocompromised because of anergy from advanced tuberculosis or concurrent viral infection may not respond positively to surveillance measures. A 90-day quarantine should allow enough time for animals to become either immunocompetent or further debilitated. For example, it is well known that nonhuman primates coming into contact with humans often acquire measles virus and are temporarily severely compromised immunologically (McChesney *et al.*, 1989). If these animals have been recently infected with tuberculosis, they may not demonstrate clinical signs or be able to respond to the tuberculin antigen. The immunosuppression lasts for several months, after which time the animals may mount a response to the tuberculin test if infected. If the animal is compromised because of anergy or one of the simian retroviruses, it may or may not respond to the test, but after 90 days in quarantine the condition of the animal should deteriorate or a variety of other disease conditions may occur. There may be instances when the quarantine period can be decreased to 60 days. For example, if there are no other primates at risk, the quarantine goal would be to assess the health status, condition the animals, and protect personnel from zoonotic diseases. Another situation may be such that all animals currently housed in the facility are used on acute nonsurvivial studies and the new animals will also be used on acute nonsurvivial studies. In this situation years of research and irreplaceable animals will not be lost if there is a problem. Decisions concerning length of quarantine should be based on professional judgment and careful analysis of risk factors. Shorter quarantine periods should be the exception rather than the rule.

Each shipment or group of animals should be housed together and not combined with other shipments or groups unless

approved by the veterinarian in charge of quarantine. If groups are combined, the quarantine period commences the day the last animal enters the quarantine room [Centers for Disease Control (CDC), 1990a]. At no time should different species be housed together in the same room during quarantine. To minimize the potential of contamination, no animal should be moved to a different cage or room during the quarantine period without prior approval from the veterinarian in charge.

G. Receiving

Nonhuman primates are transported most frequently via air for long distances and via specialized road vehicles for shorter distances. If animals have traveled a long distance, especially if coming from overseas, it is imperative that they be given food and water as soon as possible. On arrival at the quarantine facility, the transport cages should be taken directly to the assigned room via the soiled corridor or a route which prevents contaminating the facility. For safety, a minimum of two individuals should be available to transfer animals from the transport containers to the cages. The transport cages should be disinfected with chemicals or heat sterilization prior to disposal or returning to the sender. If wooden transport cages cannot be adequately disinfected, they should be appropriately discarded. State and local waste management regulations will determine how the transport containers will be classified for disposal purposes.

Once in their individual cages animals should be observed for evidence of illness by trained technicians or a veterinarian. It is important to note the behavior of the animals, including their ability to use the automatic watering system or bottles. If it is not readily apparent that the animals know how to use the watering system, a bowl of water should be provided. If animals appear dehydrated it may be helpful to offer a flavored electrolyte solution to encourage drinking and to replace electrolytes (e.g., Gatorade, Prang, or Tang with electrolytes added). If animals are noted to be ill or weak, a veterinarian should be consulted immediately.

H. Quarantine Husbandry

The quarantine rooms should only be entered when necessary; generally this is twice a day for feeding, cleaning, and animal surveillance. The room should be viewed through the observation windows prior to entering the room to ensure that no animals are loose in the room.

Prior to initiating the husbandry procedures the animals should be examined for signs of injury, lethargy, anorexia, dehydration, or other physical and behavioral problems. After the room is disturbed even the more subdued animals react to the observers presence and may mask signs of illness with a fight or flight response.

The character of the feces and urine should be noted in the cage pan prior to disposal. The waste should be disposed of carefully so as to minimize the creation of aerosols. The waste can either be hosed down the drain or, if bedding is used in the cage pans, be collected in bags for disposal. The room walls, floor, and ceiling should be carefully cleaned.

During the quarantine period, cages should be cleaned daily and removed for sanitation every 2 weeks. In the face of infectious disease outbreaks, professional judgment may dictate modifying this schedule in order to minimize stress to the animals and/or excessive exposure of personnel to infectious animals and their waste. Prior to removing cages and/or racks from the quarantine room, they should be cleaned in place and liberally sprayed with a disinfectant. The soiled cages should be taken directly to the soiled side of the cage wash area and immediately placed in the cage washer. It is helpful to replace cages on the days the animals are immobilized for routine examinations and tests. The soiled cage is removed and the immobilized animal is allowed to recover in a clean cage.

All bedding and other waste material should be double bagged and disposed of properly. This will vary based on local requirements. Most states have very specific requirements for the disposal of infectious or potentially infectious waste. In an uneventful quarantine, the waste may be washed down the drain to the sewer system or be double bagged and disposed of as other animal waste, i.e., incinerated or in a landfill. If the waste is determined to be infectious, federal, state, and local regulations should be consulted but usually they require disinfecting the waste prior to disposal either through the use of chemical or heat sterilization.

I. Quarantine Health Surveillance and Diagnostic Procedures

The specific details of health surveillance and diagnostic procedures (initiated in quarantine) are discussed later. The focus here will be how these procedures contribute to the successful quarantine program which is the cornerstone of the preventive health program for nonhuman primates.

After receipt the animals should be observed and allowed to acclimate for 48 to 72 hr. Because of the stress of shipping, animals may show evidence of disease during this time. The daily surveillance performed by trained animal technicians is critical in identifying animals in need of treatment. Confidence in the competence of the technical personnel is imperative since quarantine facilities are minimally accessed and are usually separate from the colony. A clear, reliable reporting mechanism is essential in ensuring that the observations made by the animal technicians result in timely veterinary intervention.

Following the 72-hr acclimation period, animals should be chemically immobilized and examined by a veterinarian, receive their first tuberculin skin test, have body weight recorded, and be permanently identified. The physical examination should be documented in the health record of the animal. Correction of minor health problems (e.g., dental tarter or minor

hernias) should be delayed until the end of quarantine since treatment usually involves extra personnel and equipment that increase the risk of disease transmission.

The tuberculin skin tests should be evaluated at 24, 48, and 72 hr by an individual competent in the interpretation of the test and the results recorded. The tuberculin test and body weight determination should be repeated every 2 weeks for the duration of the quarantine period, with a minimum of five consecutive negative tuberculin tests (CDC, 1993). The weight history should be carefully monitored and animals that have lost more than 20% of their body weight or young animals who are not gaining weight after 45 days should be reexamined and evaluated.

During the early part of quarantine, baseline clinical pathology samples should be collected. This allows time for repeat tests for equivocal results or for post-treatment evaluation. Complete blood count, serum chemistry, thick and thin blood smears, serum for storage and serological testing, fecal sample for ova and parasite examination, and rectal swab for enteric pathogen culture are among the tests usually performed.

Appropriate immunizations can be administered during the quarantine period but care should be taken that the immunizations do not interfere with the diagnostic testing, i.e., measles vaccine and tuberculin testing. Administering prophylactic anthelmintics and dusting for ectoparasites are performed while in quarantine.

J. Illness in Quarantine/Positive Tuberculin Test

Nonhuman primates are susceptible to a host of disease conditions, both infectious and noninfectious. The decision to treat animals that have noninfectious diseases should be based on the value of the animal because working in quarantine involves additional equipment, labor, and time. The decision to treat animals that have a potentially contagious disease must be carefully evaluated on the basis of the quarantine group, the potential risk to colony health, and personnel health and safety. For new or young animals (particularly wild caught), treatment of serious illness may not be justified. For valuable animals or for those that are transferring between institutions and have an extensive research history the risk may be justified.

Once the decision to treat is made, care must be taken to protect other animals at risk. Instruments and supplies used should be either disposable or sterilized with either steam or gas. Treatments or procedures should be performed after all other work is completed. If this is not possible, alternate policies and procedures should be established to minimize disease transmission.

Because nonhuman primates are extremely susceptible to tuberculosis, a positive intradermal palpebral test should be immediately reported to the quarantine veterinarian and decisive action taken. In most cases of an unequivocal positive tuberculin reaction the decision will be to euthanize the animal, perform a postmortem exam, and submit tissue samples for histopathology, special staining, and culture and sensitivity. Laboratories such as the *Mycobacterium* branch of the U.S. Veterinary Resource Laboratory in Ames, Iowa, are available for consultation (National Animal Disease Center). The entire shipment of animals must begin a new quarantine period.

If the tuberculin reaction is questionable and isolation is possible, euthanasia may not be necessary. The animal should be labeled a suspect and additional diagnostic tests performed (see Part A, Section V, B, 2). If the test results are still not clear, the decision to perform further diagnostic tests such as culture of bronchial washings or gastric lavage must be carefully evaluated using the risk evaluation criteria mentioned previously.

Animals that die in quarantine for unexplained reasons should be handled cautiously. Animals should be carefully double bagged, weighed, and submitted for necropsy. The veterinarian should be notified immediately and should proceed with the necropsy. If the veterinarian determines that the death was possibly due to a disease that is a public health concern or reportable, CDC and state public health agencies should be contacted for additional requirements (CFR, 1987).

K. Release from Quarantine

Prior to release of animals from quarantine all information pertinent to the group should be reviewed, including shipping documents, applicable permits, health certificates and records from the originator, tuberculin test records for each animal, individual health records containing the results of all surveillance and diagnostic procedures, and all prophylactic measures performed while in quarantine. The animals should be scheduled for a final physical examination, preferably including a thoracic radiograph, prior to being released to the colony. Following completion of quarantine, federal and state certificates of quarantine must be filed with the appropriate agency. These agencies will then forward a certificate of quarantine completion and release may proceed.

III. HUSBANDRY MEASURES CONTRIBUTING TO PREVENTIVE HEALTH

A basic component of a preventive medicine program is quality animal husbandry. High quality sanitation, appropriate environmental controls, and a nutritionally balanced diet are important for the health and well-being of a nonhuman primate colony. Facilities housing nonhuman primates should be designed and constructed to meet federal and state laws. The federal Animal Welfare Act of 1985, Public Law 99–198, establishes standards for housing, sanitation, feeding, watering, separation of species, ventilation, and veterinary care (CFR, 1985).

TABLE A.I

APPROPRIATE COMBINATIONS OF NEW WORLD PRIMATES[a]

Combination	*Aotus*	*Ateles*	*Callicebus*	*Callithrix*	*Cebuella*	*Cebus*	*Lagothrix*	*Saguinus*	*Saimiri*
Aotus	+	0		0		0	0	0	0
Ateles	0	+		0				0	0
Callicebus			+						+
Callithrix	0	0		+		0	0	+	0
Cebuella					+				
Cebus	0			0		+		0	+
Lagothrix	0			0			+	0	0
Saguinus	0	0		+		0	0	+	0
Saimiri	0	0	+	0		+	0	0	+

[a]+, safe to house together in the same room; 0, do not house together in the same room; blanks, not enough information available; do not house together unless other information can be found showing safety in housing together.

[b]*Saimiri* ssp. can be housed with *Cebus* ssp. or *Callicebus* ssp., but it may not be safe to house *Callicebus* ssp. with *Cebus* ssp. These three species should not be housed together in the same room.

TABLE A.II

APPROPRIATE COMBINATIONS OF OLD WORLD PRIMATES[a]

Combination	Asian macaques	*Cercopithecus aethiops*	*Erythrocebus patas*	*Papio* sp.
Asian macaques	+	0	0	0
Cercopithecus aethiops	0	+	/	+
Erythrocebus patas	0	/	+	/
Papio sp.	0	+	/	+

[a]+, safe to house in the same room; 0, do not house in the same room; /, *E. patas* may carry SHF virus, *Papio* and *Cercopithecus* may develop mild disease from SHF virus or may also become carriers.

Physical separation of species is recommended to prevent disease transmission, reduce anxiety due to interspecies conflict, and meet experimental requirements (DHHS, 1985). There are many examples in the literature of subclinical or latent diseases of one species of nonhuman primate causing overt disease in another species of nonhuman primate (Morita *et al.*, 1979; London, 1977). Occasionally, some species must be transported or otherwise come in contact with another species of nonhuman primate. If contact with another species is unavoidable, Tables AI and AII may be used as guides for safe combinations of some of the more commonly used nonhuman primates. Contact between Old World and New World nonhuman primates is not recommended.

A. Sanitation

Sanitation programs will vary according to the type of housing and species involved. The Animal Welfare Act requires that primary enclosures in an indoor setting be cleaned daily and sanitized biweekly. Nest boxes, perches, and enrichment devices must also be sanitized or replaced when soiled (CFR, 1985). The schedule for cleaning these items will be determined by the item and the species using it. For example, *Callithrix* and *Aotus*, which scent mark, require schedules which alternate sanitation of the primary enclosure and the enrichment device so that a portion of their environment maintains some of the territorial scent mark. This is especially important in a breeding situation (Richter, 1984).

B. Environmental Controls

The Animal Welfare Act requires that the ambient temperature of indoor facilities should not be allowed to fall below 45°F. When ambient temperatures reach 85°F, provisions should be made for cooling or increasing air movement. The recommended temperature for most primate facilities is 64°–85°F (DHHS, 1985). Species, size, and condition of animals should be taken into consideration when determining the temperature for a particular room or facility. Animals housed in outdoor facilities must be acclimated. These animals can often tolerate a wider range of temperatures than is recommended for nonacclimated animals. When temperature extremes occur, additional measures must be taken to ensure the well-being of these animals. These may include shelter from sunlight, provision of sprinkler systems, or access to bodies of water for high temperatures. Where low temperatures occur, shelter from the elements must be provided. If this is not adequate for the age groups or species involved, supplemental heat sources such as heated surfaces, nest boxes, or enclosures should be provided.

For most species a wide range of humidity is acceptable (30–70%). Some species such as marmosets and tamarins require a level of humidity of at least 50% (Richter, 1984). Indoor housing should control the humidity levels whereas the ambient humidity should be considered when designing outdoor facilities. Conditions of high humidity may exacerbate problems associated with temperature extremes.

In indoor facilities a 12-hr light and 12-hr dark cycle is generally used to provide illumination sufficient to allow normal biological and physiological responses of most species of nonhuman primates [Institute of Laboratory Animal Resources (ILAR), 1980]. Facilities housing *Aotus* or other nocturnal species may choose to provide a reverse light cycle. Red lighting during the dark phase (daytime) provides illumination for husbandry practices which then coincide with the active phase of the animal. White light provided at night encourages the normal resting phase for these animals to coincide with the quiet time in the facility. Outdoor lighting cannot be controlled and will have an effect on breeding and other physiological cycles. The spectrum and intensity of light may have an effect on the biology of nonhuman primates; however, there are no conclusive studies available at this time. The spectrum and intensity suitable for humans will probably be suitable for most nonhuman primates species. Lighting should be adequate for routine inspection and husbandry practices and should be uniformly distributed within the room.

Loud or sudden noises may be distressful to different species of nonhuman primates in a facility (ILAR, 1980). Personnel working around the animals should be educated to minimize unnecessary noise.

Bedding should be from a quality source to minimize the potential for contamination which may have a profound effect on the animals health and/or research results. Common bedding contaminants include biological and chemical agents (DHHS, 1985).

C. Nutrition and Feeding

The Animal Welfare Act requires that feed be provided which is wholesome, palatable, and of sufficient quantity and nutritive value to meet the normal daily requirements for the condition and size of the nonhuman primate (CFR, 1985). Considering the wide variety of nonhuman primates that are used in biomedical research, it is important to be aware of the differences in nutritional requirements. Diets should be designed to meet the needs of the different species in the facility. Protein requirements are age and species dependent. Certain vitamin requirements vary between species, and some groups require a high fiber level in their diet. Details on nutrient requirements of the various species are available in this book and in other sources [National Academy of Sciences (NAS), 1978].

Consistent feeding practices are an essential component of good husbandry. The quantity of food, placement within the enclosure, and time of feeding all contribute to a quality feeding program. In individual cages, feed boxes should be used whereas in social housing several feed sites should be used to ensure that adequate feed is available to all animals in the group. In outdoor enclosures, feed should be protected from the elements to preserve nutritive quality and prevent decomposition. In most situations animals should be fed at least twice daily at regular intervals to minimize digestive problems such as acute gastric dilatation (Henrickson, 1984). Conditioning animals to a regular feeding schedule may also prove useful at a later date to provide medication or as part of an experimental protocol.

Although commercial diets may provide all of the nutrient requirements of some species, other species may require supplementation to obtain optimum results. Supplements may also be used to stimulate appetites or as part of an enrichment or training program. The animal care staff may also enjoy the supplementation process. This time can be used to enhance the relationships between the staff and the animals. However, care must be exercised in the use of supplements to avoid overeating preferred food items at the expense of a balanced diet.

IV. DAILY HEALTH OBSERVATIONS

The daily detection of early signs of disease by the animal care staff is crucial to a preventive medicine program. All animals must be observed daily by trained personnel to detect signs of disease or subtle behavioral changes. These observations are reported to supervisory staff and ultimately to the veterinarian in charge of animal health care. The veterinarian must examine the reported animals and determine need for care.

Animal care personnel must be educated in the normal behavioral repertoire of the different species housed in the facility. For example, *Saimiri* spp. exhibit a greater degree of activity than *Callicebus* spp. (Mendoza, 1991). The macaques even differ among species in the degree of aggressiveness with *M. mulatta* and *M. nemestrina* exhibiting a higher degree of aggression than *M. radiata* or *M. arctoides.* Additionally, personnel working with the same group of animals on a continuous basis should become familiar with the behavior of individual animals and report changes. A rapport between the animals and the animal care staff minimizes the fight or flight reflex and therefore allows for more candid observations of behavioral changes.

During the course of daily husbandry activities, animal care staff have the opportunity to observe changes in appetite, character of feces, and any abnormality. Although any one of these changes may indicate minor problems, they may be the first sign of a more serious disease. Accurate assessment of these changes is possible with consistent daily observations and good record keeping (NRC, 1979).

Feed left in the cage may indicate something as minor as recovery from chemical immobilization or may be an early sign of a variety of problems such as dental disease, dehydration, or renal disease. Leftover feed may also be secondary to lack of adequate water, e.g., a blocked water line or lixit, which can lead to clinical dehydration. Animals experiencing pain postsurgically or from other intermittent or chronic disease processes such as endometriosis or arthritis may also exhibit poor appetite.

V. DISEASE SURVEILLANCE

The flora of nonhuman primates has not been well defined like many other laboratory animals. The flora may contain a variety of organisms pathogenic for humans and other nonhuman primates. In order to protect the health of the colony and personnel, a program of disease surveillance and prophylaxis should be designed and directed by the veterinary staff. The program should include regular physical examinations, surveillance for those pathogens which may be present, and a prophylactic program to prevent entry of other organisms.

A. Physical Examination

The frequency of the physical examination depends on the reason for the examination. Ideally, all animals should have at least an annual physical examination by a veterinarian. The need for this may vary depending on the size and intended use of the colony.

The approach to the physical examination for a nonhuman primate does not differ significantly from that in other species. The differences are in the details and these vary among species. A thorough evaluation of the mouth of the nonhuman primate should occur early in the physical examination. In macaques the presence of oral or mucocutaneous vesicular lesions may be indicative of viral shedding of Cercopithecine herpesvirus 1 (*Herpesvirus simiae* or B virus). Although an absence of lesions does not guarantee an absence of shedding, chances of shedding are increased in the presence of lesions (CDC, 1987a). One may choose to reschedule elective procedures of an animal with lesions to decrease the potential for exposure. In most nonhuman primate species the oral mucosa is a good place to evaluate mucous membrane color and capillary refill. Other oral lesions which may have an impact on the health of the animal include tarter, gingivitis, gingival hyperplasia, bleeding of the gingiva, and broken teeth. Animals identified with dental problems should be scheduled for appropriate treatment. Routine dental hygiene will prolong the healthy life of the teeth and thereby improve the quality of the animals health. Personnel should be required to wear mucus membrane protection (e.g., goggles and mask or face shield) when working in the oral cavity of macaques. Facial abscesses may also be an indication of diseased teeth. Most often the maxillary canines, whose roots are long and curved, are involved and lesions may occur as high as the orbit and even involve the eye itself. In many species the male develops very large canine teeth. These present additional risk of injury to personnel, other animals in social housing, and occasionally to the animal itself if self-trauma occurs. The teeth can be blunted while they are still growing. Once they are mature, the teeth may be cut and filled using a pulpotomy or root canal, or a submucosal vital root retention technique (Schofield *et al.*, 1991). Pulling of healthy canines is less desirable as it is difficult, it may be overly traumatic, and it may lead to deformation of the dental arcade and subsequent malocclusion.

The physical examination should also include auscultation of the thorax, abdominal palpation, and, where possible, a rectal examination. In many species the cervix and uterus are best palpated using the bimanual method with one finger in the rectum and one hand on the abdomen (Mahoney, 1975). In smaller species such as *Saimiri* and the Callitrichids, one is limited to abdominal palpation and the nonpregnant uterus may be difficult, if not impossible, to detect. Palpation of the uterus is useful in detecting abnormalities, determining pregnancy, and estimating gestational age.

Clinical laboratory tests are important adjuncts to the physical examination. For routine and preproject examinations, baseline tests are usually indicated. These might include complete blood count, chemistry profiles, and possibly serology and/or parasite examinations. When indications of disease are present, they will dictate which tests are required. Where published values exist, there is often a wide range of normals and frequently a limited number of species represented. Whenever possible one should try to work with the clinical pathology laboratory to establish colony normals for the species and particular cohorts of animals in the facility.

B. Surveillance and Diagnostic Testing

The design of the disease surveillance program will vary with the size of the colony, the species involved, and the use of the animals. The program should include baseline data and regular testing regimes.

1. Serum Banking

Baseline data should include a number of elements. One important element is the serum bank (ILAR, 1980). Serum should be collected and frozen on a regular basis (e.g., annually) for future use.

These samples may prove valuable for retrospective epidemiological studies, as a resource for investigators, and to resolve an institutional liability situation. Detailed and accurate records must be available to make full use of the serum bank. Along with the identification and health status of the animal, one should be able to track the location of the animal and what other animals it was exposed to at the time of collection. Other important information to have as part of the baseline data might include CBC, serum chemistry, parasite screening, and rectal culture.

2. Routine Tuberculosis Screening

Tuberculosis is a highly contagious, fatal disease in nonhuman primates, especially Old World monkeys. The disease in nonhuman primates is usually caused by *Mycobacterium tuber-*

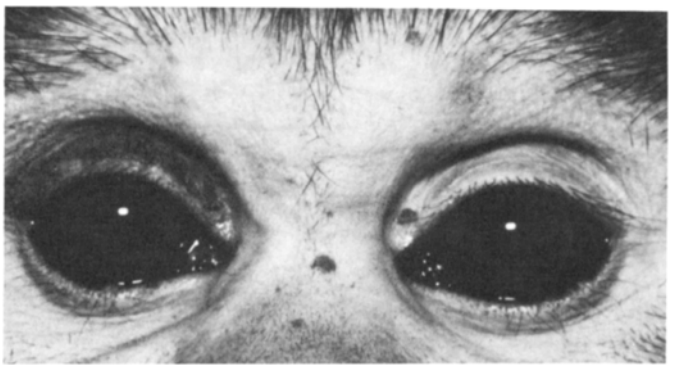

Fig. A.1 A grade 1 tuberculin test reaction. Bruising of the right eyelid caused by injection of tuberculin is shown. A grade 1 tuberculin test reaction, bruising, is considered negative.

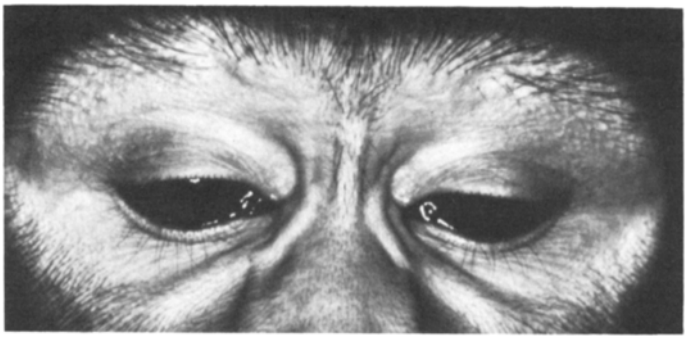

Fig. A.2 A grade 2 tuberculin test reaction. Various degrees of erythema of the palpebrum without swelling are demonstrated. A grade 2 tuberculin test reaction is considered negative.

culosis or *M. bovis*. The most common method of testing is the intradermal injection of mammalian tuberculin at the edge of the upper palpebrum. A positive test results in swelling of the lid because of the delayed hypersensitivity response. A negative response indicates the animal has not been exposed to tuberculosis, has not had time to mount an immune response, or is incapable of mounting an immune response due to immunosuppression. Immunization and subsequent testing with tetanus toxoid may aid in predicting anergic animals (Davis *et al.*, 1988; Corcoran and Jaax, 1991). Mammalian tuberculin, which is less purified but has more tuberculin units than PPD (the product most often used in human dermal testing for tuberculosis (CDC, 1990c)), is used in nonhuman primate species as PPD may not elicit a strong enough response to identify infected animals. The tuberculin test is read at 24, 48, and 72 hrs by observing the palpebrum for swelling and erythema. The palpebrum is the preferred site of testing because of the ease of observation without the need for restraint. A record should be kept of which palpebrum is used and subsequent tests are generally alternated between the two sides. The intradermal injection may also be made in the skin of the abdomen. This site allows for palpation and/or measurement of the reaction for induration and is often used when retesting an animal with a questionable intrapalpebral test or as part of the baseline testing in quarantine. The site of the abdominal test should be outlined with ink for ease of reading.

The tests are graded one to five with one and two being negative, three being questionable, and four and five being positive (Figs. A.1–A.5). Equivocal tests should be repeated using the abdominal site for additional interpretation. No animals in a group where any animal is questionable or positive should be moved or exposed to new animals until test results are confirmed. Additional diagnostic methods such as gastric or bronchial lavage, culture, and radiographs may be helpful in reaching a definitive diagnosis. Some animals may have had exposure to atypical mycobacterial organisms causing cross reactivity. If *Mycobacterium avium* is a problem in a given area or group of animals, avian tuberculin can be used to identify those animals with significant response to that agent. These interpretations should be made cautiously and with careful consideration of the general health and exposure history of the animal. In at least one facility where chimpanzees were housed in indoor/outdoor compounds, several animals with histories of intermittent mild reactions to tuberculin testing, no signs of clinical disease, and negative thoracic radiographs were later found to be infected with *Mycobacterium leprae* (P. L. Alford, personal communication, 1992; Gormus *et al.*, 1991). If any animal in a group proves to be positive, that animal should be removed and all animals in the group should be immediately quarantined. The testing regime is the same as that used in an initial quarantine, with testing every 2 weeks until all remaining animals test consecutively negative for the duration of quarantine. Because of the magnitude of the risk to the rest of the colony and the difficulty in successfully treating the disease, positive animals are generally euthanized. If the value of the animal warrants treatment, a multidrug regime (e.g., isoniazid, rifampin, and ethambutol) should be used, after culture and sensitivity, for 9 to 12 months (Wolf *et al.*, 1988). The risk to the colony, the expense and difficulty of the treatment, the stress to the animal, and the impact of these potentially toxic drugs on any research protocols should be carefully evaluated before therapy is considered.

The frequency of tuberculin testing is determined by several factors. During quarantine, animals are generally tested every 2 weeks until a group has at least five consecutive negative tests. In the established, closed colony, the testing frequency is determined by the research goals, value of the colony, and the risk of exposure. Where increased risk factors occur, such as new animals being introduced, significant human exposure, or exposure to other species which may carry the organism, quarterly testing is recommended. In a closed, stable colony with limited exposure to humans, testing frequency may be relaxed to semiannual or annual.

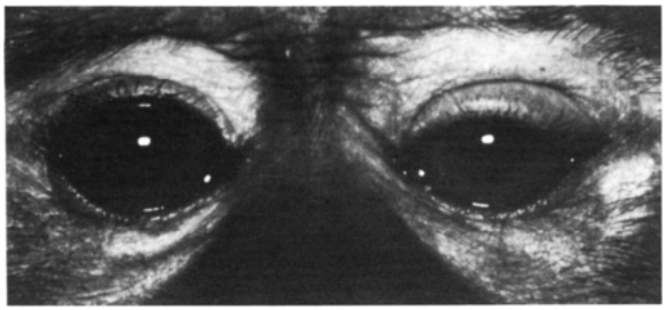

Fig. A.3 A grade 3 tuberculin test reaction. Varying degrees of erythema of the palpebrum with minimal swelling or slight swelling without erythema are shown. A grade 3 tuberculin test reaction is considered questionable.

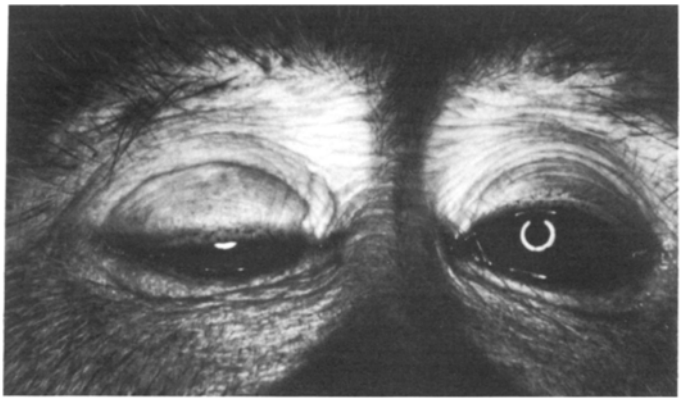

Fig. A.4 A grade 4 tuberculin test reaction. Obvious swelling of the palpebrum with drooping of the eyelid and varying degrees of erythema can be seen. A grade 4 tuberculin test reaction is considered positive.

3. Other Microbiological Disease Surveillance

a. BACTERIA. Nonhuman primates are subject to infection with a wide variety of bacterial organisms. Although it is not practical to culture all clinically normal animals for all potential pathogenic bacteria, it may be desirable to screen for some of the more commonly occurring ones, especially in those animals expected to experience stress, e.g., in quarantine, during weaning, during formation of social groups, during shipping, or in an experimental protocol. Some of the more common enteric organisms are *Campylobacter* sp., *Shigella* sp., *Yersinia enterocolitis, Y. pseudotuberculosis*, and *Salmonella* sp. *Campylobacter* and *Yersinia* require special culture techniques.

b. RETROVIRUSES. Retroviral infections may be a significant cause of disease, useful animal models, or confounding subclinical factors in research. Different species are susceptible to different types and strains of retroviruses with variable clinical presentations. In colonies with susceptible species, screening programs should be established to identify infected animals since inapparent carriers have been recognized as a problem (Lerche, *et al.*, 1986). Among the Asian macaques, the most common naturally occurring retroviruses are those of the type D group. These are often referred to as the simian retroviruses and are represented by at least five serotypes. Some of the viruses in this group can cause immunosuppression and hematological abnormalities, and may result in significant morbidity and mortality. Because these viruses require direct contact for transmission, they spread slowly in groups of individually housed animals, but can cause epidemic disease in naive group-housed populations (Lerche *et al.*, 1987). Testing for these viruses requires both virus isolation and serological screening (Lerche *et al.*, 1991). Ideally, susceptible animals should be screened in quarantine and only negative animals should be admitted to the colony. However, many facilities already have a significant percentage of viral and/or antibody positive animals. These facilities will require serial testing at 6- to 12-week intervals with removal or segregation of positives, and retesting of negatives until all animals in a group remain negative for at least two consecutive tests and possibly more in group-housed situations (Lerche *et al.*, 1991).

c. MEASLES. Measles is a highly contagious disease of human caused by the paramyxovirus, rubeola. In many species of nonhuman primates the virus causes a disease very similar to the one seen in human (T-W-Fiennes, 1972). The disease can be severe with the typical erythematous rash and secondary infections (e.g., abortions and giant cell pneumonias) due to the temporary but significant immunosuppression. However, it may also have a mild or subclinical presentation causing it to be overlooked or misdiagnosed. Many animals without a clinical history of measles may have positive antibody titers for the virus. It is desirable to know the antibody status of the animals in a colony because this zoonotic disease may be clinically significant and/or interfere with research. Serum antibody titers can be obtained from commercial laboratories offering such tests for humans. In many colonies it may be easier and more economical to rely on a prophylactic vaccination program (Part A, Section VI, B), using serum antibody tests only in those animals

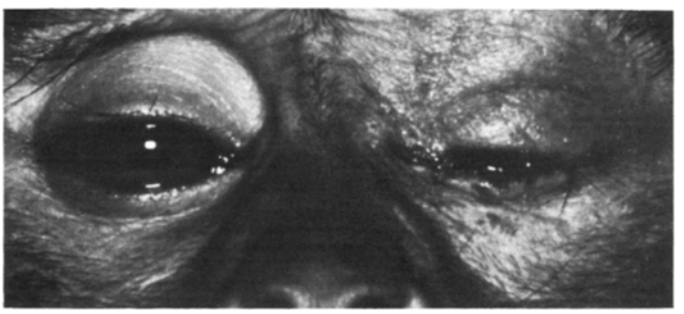

Fig. A.5 A grade 5 tuberculin test reaction. Swelling and/or necrosis of the eyelid is shown. A grade 5 tuberculin test reaction is considered positive.

that need to be maintained antibody negative for research purposes or as diagnostic aids during suspected measles outbreaks.

d. HERPESVIRUSES. The Herpesviridae is a large family of viruses which infect many animals. Among the primates there are several members worth noting. Most species have their own cytomegalovirus, a member of the subfamily Betaherpesvirinae. These are generally not clinically significant in animals with normal immune function, but may have an impact on some types of research. In most instances these will not be screened for in a general colony setting.

Members of the Gammaherpesvirinae subfamily, such as Ateline herpesvirus 2 (*Herpesvirus ateles*) (Hunt *et al.*, 1972) and Saimirine herpesvirus 2 (*Herpesvirus saimiri*) (Hunt *et al.*, 1970), are lymphotropic viruses which frequently cause latent infections but may be oncogenic and are capable of causing significant disease in aberrant host species. *Herpesvirus tamarinus* causes high morbidity and mortality in marmosets and owl monkeys (Morita *et al.*, 1979). The disease is pantropic with hemorrhage, focal necrosis, and ulceration throughout most organs and the gastrointestinal tract (Lehner, 1984). Although these viruses are not often screened for outside of specific protocol requirements, it is important to be aware of their potential and house susceptible species separately.

Herpesvirus simiae or Cercopithecine herpesvirus 1 (Herpes B virus) is a member of the Alphaherpesvirinae subfamily, genus *Simplexvirus*, and closely related to human herpesvirus 1 (HSV 1) and human herpesvirus 2 (HSV 2). B virus occurs naturally in nonhuman primates of the genus *Macaca* (Weigler *et al.*, 1990). In immunocompetent animals the clinical presentation is generally mild to subclinical. Common lesions include oral and/or mucocutaneous vesicles or ulcers and occasionally conjunctivitis. Animals remain chronically infected but only shed virus periodically (CDC, 1987a). Many colonies of macaques have endemic B virus infection with no apparent impact on the general health of the animals. Animals are generally seronegative at birth and the percentage of seropositive animals in a group increases by age cohort.

The greatest concern over B virus infection is the zoonotic risk it presents to humans. Although human infection is rare, if untreated it frequently results in a fatal encephalitis (Palmer, 1987; CDC, 1987a). Facilities where macaques are held should have bite/wound kits and instructions for wound management readily available. Employees must be made aware of the risks associated with bites, scratches, and contaminated wounds and should be instructed in the proper on-site management of these exposures. Laboratory personnel should practice universal precautions. All exposures should be referred to the occupational medical service. The physician responsible for occupational medical services should be cognizant of this virus and must keep abreast of current recommendations.

e. MISCELLANEOUS VIRUSES. A number of other significant viruses can infect nonhuman primates (Kalter and Heberling, 1990). Examples include simian hemorrhagic fever, the hepatitis viruses, the filoviruses, tetanus, and poliovirus. These may not be part of routine surveillance program because of the low frequency of occurrence, absence of infection without clinical disease, or lack of clear information on the significance of serological tests. Some of these are covered in Part A, Section VI, B, on prophylaxis.

4. Parasitic Disease Surveillance

Control of parasitic infections within a colony is based on knowledge of life cycles. The approach to interrupting life cycles depends on whether the cycles are direct or indirect. Indirect life cycles require intermediate hosts. If these are not available or can be eliminated, the life cycle and further transmission can be interrupted. Direct life cycles may be broken by a combination of anthelmintic therapies and rigorous sanitation practices. When animals are individually housed (e.g., in quarantine), the interruption of direct life cycles is more easily accomplished. Whenever possible, animals should be treated for and cleared of parasites before social housing is initiated.

The intensity of a parasite surveillance program will vary with the species of nonhuman primates, their source, and the management and housing practices. The choice of anthelmintics will be dictated by the agents identified in the surveillance program.

a. MALARIA. Nonhuman primate malaria is not uncommon in animals acquired from areas where malaria is endemic. Since the intermediate host is not present in the laboratory, transmission by this method is not a problem; however, transmission may occur directly through blood transfusion, congenital infection, or percutaneous inoculation. If treatment is required, the current CDC recommendations for presumptive pediatric treatment of malaria should be followed (CDC, 1990d).

VI. DISEASE PROPHYLAXIS

A. Designing a Program

The decision to prophylactically immunize nonhuman primates will depend on many factors as it does in humans (CDC, 1991c). Several factors to consider when designing an immunization program for nonhuman primates are species susceptibility, ease of transmission (e.g., colony risk), safety, cost and known efficacy of the prophylactic agent, cost of treatment (time and materials), and research interference. Management procedures may influence the need for prophylaxis. Facility design and policies which dictate personnel immunizations, education, and restriction of visitors may be used to decrease the need for some nonhuman primate immunizations.

Recommendations and/or current practices for immunizations of nonhuman primates vary widely among facilities. Many primate veterinarians rely on human pediatric recommendations

when designing immunization programs. These recommendations can be found in the CDC-MMWR "Recommendations and Reports." All references in this section to MMWR refer to the human recommendations in these publications.

B. Specific Vaccines to Consider

1. Tetanus

New World and Old World monkeys and apes are susceptible to tetanus (*C. tetani*) infection. Without costly and time-consuming therapeutic intervention, significant mortality results (Goodwin *et al.*, 1987). Vaccination is recommended for outdoor-housed animals where the risk of tetanus infection is significant. The risk for contracting this disease will decrease if the animals are housed indoors. However, fighting in indoor group-housed animals, resulting in significant wounds, may also result in a risk for tetanus. Tetanus in an individual does not present a risk to the rest of the colony as do other contagious diseases.

2. DPT

The diphtheria, pertussis, and tetanus (DPT) immunization has been used historically for infants of the great ape species to prevent these diseases. However, since the introduction of the vaccine in the 1940s, there has been a marked decline in the number of human cases of diphtheria and pertussis reported. These diseases are no longer considered common major causes of childhood morbidity and mortality. Concurrently the threat to infant great apes from humans has also decreased. In addition, there are potential complications reported in humans from the pertussis component of the vaccine (CDC, 1991b). Significant side effects have also been noted in at least one infant chimpanzee after administration of the DPT vaccine (P. L. Alford, personal communication, 1992). Management policies mandating demonstration of adequate childhood vaccination for employees and prohibiting children from having animal contact will further protect animals at risk. Given these facts, the risks of vaccination would appear to outweigh the benefits of this vaccine in most nonhuman primate programs.

3. Measles

Measles is not a naturally occurring disease of nonhuman primates (T-W-Fiennes, 1972). It is highly transmissible between human and monkeys as well as among monkeys. In New World monkeys, infection may cause serious disease. In healthy groups of Old World nonhuman primates the disease does not usually result in significant morbidity, although it does result in temporary but severe immunosuppression. In high risk groups of nonhuman primates (e.g., pregnant females, nursery-reared infants, and geriatric colonies), measles may result in significant morbidity (J. A. Roberts, personal communication, 1992). Although the incidence of this disease is decreasing, there are still age cohorts of individuals that may have received inadequate long-term immunity from childhood vaccinations (CCD, 1989a). These individuals may still be susceptible and able to transmit the disease. Subclinical cases of measles in nonhuman primates may interfere with certain types of research, particularly involving the immune system. Human vaccines are available (e.g., Merck, Sharp, Dohme; Connaught Laboratories) and are currently in use but are relatively expensive for use in large colonies. The canine vaccine (Norden) for use in puppies to prevent canine distemper is much less expensive. A preliminary trial using this product in rhesus monkeys showed stimulation of an antibody response without negative side effects (Staley *et al.*, 1995). Challenge trials have not been undertaken at this time.

4. Polio

Historically, vaccination was routinely recommended for the medical management of great apes (Keeling and McClure, 1972). Vaccination with oral polio was practiced according to human infant recommendations (CDC, 1987b). With the decrease in incidence of polio in the general population and the high percentage of individuals having a history of polio vaccination, routine vaccination may not be warranted except in certain high risk circumstances.

5. *Haemophilus influenzae*

Haemophilus influenzae is primarily a problem in great apes. Infection often results in septic meningitis and is expensive and time-consuming to treat. The ease of transmission and the seriousness of the disease in humans have resulted in recent CDC guidelines to amend the human immunization recommendations (CDC, 1991a). In a valuable colony of great apes, immunization with the *Haemophilus* b vaccine according to the CDC recommendations for infants and children is probably warranted.

6. *Pneumococcus*

Pneumococcus pneumonia is a problem primarily in Old World monkeys and apes. Serological surveys have indicated that large numbers of nonhuman primates are seropositive for pneumococcus but do not exhibit clinical disease unless there are other concurrent problems (e.g., stress, inclement weather or viral respiratory infections) (Jones *et al.*, 1984). During an outbreak, high morbidity and mortality may exist in high risk groups (e.g., infants and older animals). Short-term prophylaxis with long-acting penicillin may decrease morbidity during an outbreak in unvaccinated animals. Treatment is expensive and time-consuming, and immunization with pneumococcal polysaccharide may be warranted in valuable populations with significant risk (CDC, 1989b).

7. Hepatitis A

Hepatitis A virus infection has been reported in New World and Old World monkeys and great apes (Deinhardt, 1976). Clinical signs vary among the species and among different individuals. The fecal–oral transmission of this agent may be a problem in group-housed individuals. Preexposure prophylaxis is not usually required but postexposure prophylaxis with immune globulin, especially during the incubation period (15 to 50 days), may be protective against clinical illness (CDC, 1990b).

8. Hepatitis B

Hepatitis B is a naturally occurring virus of humans. Experimentally induced hepatitis B virus has caused clinical disease in chimpanzees and white-handed gibbons. A possible case of naturally occurring hepatitis B has been reported in cynomolgus monkeys (Kornegay *et al.*, 1985). Transmission of this virus is usually through contaminated blood and serous fluid and is not easily accomplished. Vaccination is not usually performed in chimpanzees since many of these animals are used on hepatitis B research projects precluding vaccination. There are an estimated 750,000 to 1,000,000 chronically infected human carriers of the hepatitis B virus. Human transmission from health-care workers to patients has been documented in invasive procedures (CDC, 1990b). In light of this information, it seems likely that the possibility exists for animal care staff to transmit this virus to susceptible nonhuman primates through contact with infectious blood or body fluids. The antibody and antigen status of individuals working with these animals should be known both for the protection of the individuals and the animals they work with. Susceptible individuals should be offered the vaccine.

9. Rabies

Although nonhuman primates are susceptible to the rabies virus it does not usually present a problem under most laboratory animal conditions. A known exposure of four gibbons and postexposure vaccination has been reported (Smith *et al.*, 1987). Although the postexposure treatment appeared to be successful and the animals were maintained in strict isolation, it was not without risk to personnel and other animals. Vaccination should be considered in outdoor-housed animals in endemic rabies areas because of the inability to treat rabies and the risk to humans in contact with a rabid animal.

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PART B. COLLECTION OF BIOLOGICAL SAMPLES AND THERAPY ADMINISTRATION

Robert C. Dysko and Darrell E. Hoskins

I. INTRODUCTION

The information presented in this section is intended to assist the veterinarian in determining the most suitable method to obtain samples for disease diagnosis and to provide systemic therapy in the nonhuman primate. Proper collection of fluid and tissue samples and proper administration and delivery of medications are significant components of maintaining the health of nonhuman primates. These techniques can also be used to collect data for biomedical research purposes. In some cases, the use of specific fluid and tissue collection techniques in the live animal may eliminate the need to kill a nonhuman primate to harvest research samples, thus conserving the number of nonhuman primates used in research.

Fluid and tissue sample collection must be approached judiciously, with full understanding of the advantages and limitations of each technique. Proper care and attention must be paid during sample collection with regard to the specific assays which will be performed on the sample and whether the assays require special handling of the sample (e.g., buffered media). Sterility must be maintained for all samples which are being obtained for microbiological culture, as well as for all samples which require invasion of the integument barrier, such as tissue biopsies. Consideration must be given to the stress that an animal might endure during the procedure and whether the end justifies the means. The limitations of each tissue sampling technique should always be given just consideration; whereas percutaneously obtained biopsy specimens may be faster and easier, they do not allow direct visualization of the organ to be sampled.

It is very important to remember that fluids and tissues from nonhuman primates can harbor zoonotic pathogens, some of which can cause fatal disease in humans (e.g., simian herpesvirus, *Mycobacterium tuberculosis*). Proper safeguards should be taken by all personnel involved in the handling of nonhuman

primate fluids and tissues, from the individuals performing the sample collection to the individuals processing the samples in an assay. One need only remember that it was the laboratory workers that processed the tissue samples who were fatally infected with the Marburg virus in 1967 (Acha and Szyfres, 1980). Laboratory-associated transmission has also been suspected in several cases of human *Herpesvirus simiae* (B virus) infection (Kaplan *et al.*, 1988). Biohazards associated with nonhuman primates are detailed in Chapter 15.

Anesthesia of nonhuman primates is discussed fully in another Part C of this chapter. It is recommended that for most of the procedures discussed here that dissociative anesthesia supplemented with a tranquilizer or analgesic should be used for the well-being of the patient and for the safety of the veterinary staff.

In Part B, Section III on tissue collection, several organs are not specifically mentioned (e.g., urinary bladder, pancreas, adrenal gland). Techniques for biopsy of these organs in nonhuman primates were not located in the literature. For such organs and tissues, techniques described in the general veterinary literature, or for human pediatric patients, should be considered and modified where appropriate.

II. FLUID COLLECTION

A. Blood

Prior to harvesting blood from nonhuman primates, it should be determined that the amount of blood to be withdrawn will not be detrimental to the health of the animal; this is especially important in the smaller nonhuman primates (less than 3 kg). McGuill and Rowan (1989) reviewed the literature with regard to the effects of blood loss in several animal species, and provided some general recommendations for "safe" sampling amounts. Although guidelines for blood sampling in nonhuman primate species were not specifically addressed, the general philosophy of limiting sampling amounts, replacing fluid losses, and waiting for proper recovery of the hematopoietic system before rebleeding should be adopted. Two general philosophies presented by McGuill and Rowan regarding maximal one-time (nonmultiple) blood samples are a maximal sample size of 15% of the blood volume, and the 10%–10% rule, which states that the maximal blood sample size is 10% of a blood volume estimated to be 10% of the body weight (which equals a sample of 1% of the body weight).

1. Acute Venipuncture

The most common site for blood sampling in the nonhuman primate is the femoral vein. Other sites include the jugular, coccygeal, and lateral saphenous veins (Bivin and Smith, 1984). Typically a 5.0- or 10.0-ml syringe with a 20- to 23-gauge needle works best for obtaining percutaneous blood samples from macaques (3–15 kg). Butterfly needles are advantageous for lateral saphenous venipuncture since the needle and hub stay flat to the skin surface of the animal. For squirrel monkeys, the femoral or jugular vein is the recommended site for sampling of blood, using a 0.5-inch, 20-gauge needle (Dukelow and Asakawa, 1987). Venipuncture in marmosets is best accomplished in the upper femoral vein, using a 25- or 26-gauge needle and a 1.0-ml syringe (Hearn, 1987). The Vacutainer blood collection system (Becton-Dickinson, Rutherford, NJ) can be utilized in larger nonhuman primates, such as baboons and great apes, but the negative pressure created by the collection tube can collapse the veins in smaller species. Adequate pressure should always be applied to the venipuncture site after withdrawal of the needle to ensure proper hemostasis. This is especially critical in owl monkeys, which can have high circulating levels of antithrombin III, and thus are prone to hemorrhage and hematoma formation (Loeb *et al.*, 1976).

Anesthesia is not required for most venipunctures. If the monkey can be removed from its primary enclosure without difficulty it is possible to obtain a quality blood sample simply with manual restraint. Light sedation (ketamine at 5 mg/kg) may be helpful if the animal is fractious.

Nonhuman primates can be trained to present their arms or legs for venipuncture. This may require cage modifications to permit exteriorization of the limb, but such training results in the ability to obtain repeated blood samples without the interference of capture distress or anesthesia to either the blood analyses (Reinhardt, 1991) or the daily routine of the animal. This system is especially efficacious when multiple small samples are needed over an extended time period which would exceed the typical life span of a chronic indwelling catheter (e.g., glycemia checks for diabetic animals). Vertein and Reinhardt (1989) detailed the methods that they used to train female rhesus monkeys to present their legs for in-home cage venipuncture of the saphenous vein. The monkeys were housed in pairs in restraint-back cages, and all eight monkeys were trained within 24 workdays. The authors felt that the presence of a cage mate facilitated the training operation. Reinhardt *et al.* (1990) also found that paired animals which were sampled in their home cage had significantly lower elevations in serum cortisol (as an indicator of a stress response) when compared to single animals either sampled in their home cage or placed in a transport box and restraint apparatus. Phillippi-Falkenstein and Clarke (1992) described a program for training corral-housed rhesus monkeys to present their legs for saphenous vein bleeding. The training regimen involved chute transfer to a holding cage, secondary transfer to a sample collection cage, and limb presentation. The authors felt that consistency of procedure and positive reinforcement for cooperative behavior were the keys for the success of this 39-day training regimen.

2. Venous Catherization

Indwelling catheters are useful for those animals in which multiple blood samples (and/or infusions) are required for a

period of time which precludes constant anesthesia or are of fairly large volumes within a short period of time. Many different systems have been devised for chronic catheterization of nonhuman primates. Bree *et al.* (1982) described the acute implantation of a 22-gauge catheter (Intracath, The Deseret Company, Sandy, UT) into the saphenous vein of a lightly sedated rhesus monkey. The catheter was attached to a larger diameter silicon tube, and the combination was secured to the leg with elastic tape. The leg was immobilized in a splint, and the monkey was allowed to recover in a primate restraint chair. The catheter was used to withdraw small aliquots of blood over a 4- to 6-hr period, and then was removed. Bedford *et al.* (1977) described a surgical cut-down technique for placement of a catheter into the vena cava via the saphenous vein. Again, the duration of successful catheterization corresponded with the ability to maintain the animal in a restraint chair. Epstein and Chez (1976) inserted catheters into the umbilical artery of newborn rhesus monkeys in order to obtain serial blood samples over an 8-hr period.

Techniques for the placement of chronic indwelling catheters in superficial veins have been described. A long-term saphenous vein catheter was successfully placed in a rhesus monkey using a lightweight casting system to immobilize the leg and thus allow the animal to return to its home cage postsurgically (Conti *et al.*, 1979). Jacket and backpack systems for the maintenance and protection of surgically implanted vascular catheters have been described for marmosets (O'Byrne, 1988), rhesus monkeys (Herndon *et al.*, 1981), and cynomolgus monkeys (Scalese *et al.*, 1990). Jacket and tether systems for the implementation of chronic catheters in nonhuman primates have been described by Bryant (1980), McNamee *et al.* (1984), Ducsay *et al.* (1988), and Coelho and Carey (1990). The use of these advanced chronic indwelling catheter systems is discussed further in Part E of this chapter entitled "Special Techniques."

3. Arterial Blood Sampling

Arterial blood sampling may be indicated for certain diagnostic or experimental purposes, such as blood–gas analysis. Although percutaneous sampling from the femoral artery is possible, it is recommended that visualization of the artery be accomplished by a surgical incision prior to puncture. This guarantees the retrieval of arterial blood and allows for proper hemostasis at the puncture site. Chronic catheterization is also recommended when multiple arterial blood samples are required. A technique for femoral artery catheterization that precludes ligation of the artery distal to the insertion site (a common practice) has been described by Lindsey *et al.* (1990).

4. Fetal Blood Sampling (Cordocentesis)

Access to fetal or placental blood prior to parturition can be accomplished using either laparoscopy/fetoscopy or interventional ultrasound. Davidson *et al.* (1978) described a laparascopic technique for obtaining fetal/placental blood in several species of Old World monkeys. Morris *et al.* (1980) utilized ultrasound to visualize the placenta, and then penetrated the nonplacental uterine wall with a fetoscope that had blood sampling capabilities. In both of these studies blood was obtained from the fetoplacental vasculature, and admixture with either amniotic fluid or maternal blood was a common complication.

More recent publications (Lindgren and Lindberg, 1985; Tarantal, 1990) described the use of ultrasound guidance for percutaneous umbilical cord sampling (cordocentesis) in macaques. The ultrasound was used to visualize the placental discs, fetus(es), and umbilical cord(s). Tarantal (1990) recommended a 25-gauge, 3-inch spinal needle, inserted percutaneously into the uterus. The location of the primary placental disc determined if the needle penetrated the disc itself to obtain blood from the base of the umbilical cord or traversed the amniotic space to penetrate the cord more distally. Fetal heart blood could also be sampled with this technique if the umbilical cord was inaccessible. Lindgren and Lindberg (1985) described a similar technique to obtain blood from the fetal intrahepatic umbilical vein of rhesus monkeys.

B. Urine

1. Free Catch

The simplest method for obtaining a free-catch urine sample from a nonhuman primate is to cage the animal individually and collect the urine from a clean litter plan. The advantages of this system are that it requires a minimum of manpower and the animal does not have to be manipulated. However, the sample is rarely without contamination from food, drinking water, feces, or other debris. Several litter pans have been designed to separate the fluid waste from any solids, usually by fashioning catch screens, slopes, and/or drains into the design of the pan (Stoller *et al.*, 1971; Suzuki *et al.*, 1989). Modified rodent metabolic cages have been used to obtain 24-hr (and longer) urine samples from marmosets (Lunn, 1989) and owl monkeys (Weller *et al.*, 1991). Advanced electromechanical systems have been designed to collect and segregate free-catch urinations from caged chimpanzees (Stoller *et al.*, 1971). It may also be possible to train nonhuman primates to urinate for positive reinforcement (similar to limb presentation for blood sampling).

Manual restraint of a nonhuman primate may induce urination, and thus permit collection of a fresh, uncontaminated sample. A flexible, silicone sheath designed by Rahlmann *et al.* (1976) was attached to the penis of pig-tailed macaques; it permitted collection of free-catch urine from temporarily chaired animals. The authors commented that soft latex surgical drains (Penrose drains) might also work in a similar fashion for short periods of time.

A modification of the disposable diaper method used for urine collection in human children was made by Lopez-Anaya *et al.* (1990) for infant pig-tailed macaques. The diaper con-

sisted of absorbent cellulose sponges (Spontex, Spontex Inc., Columbia, TN), polyethylene sheets cut to hold the sponges in a diaper shape (with an exit hole for the tail), and Velcro straps to secure the diaper around the animal. The diapers were changed every 2–3 hr during the study.

Another human pediatric urine collection technique is the use of a urine collection bag adhered to the perineum (Glass, 1986). The patient is monitored carefully after the bag is placed, and the urine is retrieved as quickly as possible after micturition. This technique could be modified for use in tractable (or partially sedated) nonhuman primates, but could not be relied on for microbiological culture because of urine contact with the skin and hair.

2. Urethral Catheterization

Urethral catheterization is possible in both male and female macaques, baboons, and chimpanzees, but requires an aseptic technique and either chemical or physical restraint. Female macaques can be readily catheterized with a typical 3.5 French "Tomcat catheter." For urethral catheterization the animal is best positioned in ventral recumbency on an examination table, with its legs suspended over the table edge. Visualization of the urethral papilla may require the use of a vaginal speculum, although in some animals it can be seen readily when the animal is positioned properly. Bahnson *et al.* (1988) reported a similar technique using 7 and 9 French catheter sheath introducer systems with guidewires. Success in catheterizing the urethras of male macaques seems to be variable and operator dependent. Males require a more flexible catheter than females so that it can pass around the flexure at the pubic bone. When long-term urethral catheterization is required (e.g., studies with intravenous fluid infusion), appropriately sized Foley catheters can be used.

3. Cystocentesis

Cystocentesis is possible in macaques, but it is difficult because of the relatively small size of the urinary bladder. Even when distended, the bladder can be difficult to palpate because it still lies within the pelvic canal. Keeling and Wolf (1975) described a technique for cystocentesis in the rhesus monkey. The skin over the suprapubic area was shaved and prepared aseptically to prevent possible contamination of the urinary bladder. A sterile 1.5- to 2-inch long, 20- to 22-gauge needle was inserted 2 cm cranial to the pubis on the midline, and directed caudally at a 45° angle to the brim of the pelvis (toward the sacrum). The needle was advanced gently until urine appeared in the syringe, either by spontaneous flow or by aspiration. The same technique has been reported for squirrel monkeys using a 0.5-inch, 20-gauge needle (Dukelow and Asakawa, 1987). Other suprapubic aspiration techniques used for human children (Glass, 1986) might also be applicable for nonhuman primates.

C. Cerebrospinal Fluid

1. Suboccipital Puncture

Cerebrospinal fluid (CSF) is typically collected from either the cisterna magna or the lumbar subarachnoid space. Geretschlager *et al.* (1987) detailed collection of CSF from the common marmoset via suboccipital puncture. After anesthetization of the marmoset and aspetic preparation of the skin surface, the head was held in complete flexion. A 0.5-mm-diameter, 14-mm length cannula from a disposable scalp vein set was used. The cannula was inserted 8 mm distal to the external occipital protuberance and advanced until it touched the occipital bone. The cannula was then redirected toward the posterior atlantooccipital membrane, which is pierced to enter the cisterna magna. CSF flowed readily and a 1.0-ml syringe was used to gently withdraw the fluid sample. This procedure was repeated multiple times in each animal without complication. A similar approach using a 22-gauge, 1.5-inch spinal needle was performed in cynomolgus monkeys (Lipman *et al.*, 1988). In a report which compared CSF characteristics from suboccipital and lumbar puncture sites in rhesus monkeys (see Lumbar Puncture), the animals were placed in ventral recumbency, and a sterile 22-gauge hypodermic needle (length not noted) was inserted just rostral to the arch of the atlas on the dorsal midline (Smith and Lackner, 1993). In dogs and cats, the recommended site for cisterna magna puncture is the intersection of a transverse line connecting the wings of the atlas with the dorsal midline posterior to the occipital protuberance (Shores *et al.*, 1985). Use of these bony landmarks may be helpful in CSF taps performed in other species of nonhuman primates.

2. Lumbar Puncture

Lumbar puncture for CSF collection is best accomplished in anesthetized patients placed in lateral recumbency, although it has been done successfully in chaired, slightly sedated rhesus monkeys (Snead and LaCroix, 1977). The trunk of the recumbent animal should be placed in slight flexion to widen the dorsal aspect of the intervertebral space and thus facilitate penetration of the interarticulate ligament. Typically an 18- or 20-gauge spinal needle is inserted into the vertebral interspace which lies in the transverse plane with the iliac crest. In a report on lumbar puncture in the baboon, the needle was inserted one intervertebral space cranial to this transverse plane, which corresponded to the L_2–L_3 intervertebral space (Butler and Wiley, 1971). In a report on the same procedure in chimpanzees, the site chosen was the intervertebral space caudal to this transverse plane (Derwelis *et al.*, 1970).

Smith and Lackner (1993) performed lumbar puncture in rhesus macaques with the animals in ventral recumbency, using a 22-gauge needle (length not noted) inserted on the dorsal midline at either the L_4–L_5 or L_5–L_6 intervertebral space. Their report compared CSF characteristics from lumbar and

suboccipital puncture sites. Lumbar CSF had higher concentrations of total protein, albumin, and IgG, and lower concentrations of potassium and glucose when compared to CSF obtained by suboccipital puncture. The elevated concentration of protein components in lumbar samples has also been observed in humans, and it has been suggested that there is a slower rate of protein removal from the CSF in the lumbar region.

D. Oral Fluids

Salivary secretions can readily be obtained from nonhuman primates anesthetized with ketamine. The administration of parasympathomimetic agents, such as pilocarpine, can be used to increase saliva production.

The most significant need for obtaining samples of oral secretions is for determination of the possible presence of herpesvirus B in incidents of bites to human personnel. The suspect macaque should be anesthetized, and a sterile cotton-tipped applicator should be passed for a full 360° within the oral cavity for a proper buccal culture. The applicator tip should be inserted in appropriate transport media and shipped on ice for viral isolation. Conjunctival (both eyes) and urogenital swabs are also recommended from monkeys suspected of shedding herpesvirus B.

E. Respiratory Fluids

1. Pharyngeal Swabs

The collection of pharyngeal fluids for bacterial culture from nonhuman primates has been detailed by Snyder and Soave (1970). In order to protect the culture swab from oral contamination, it was passed through a modified tuberculin syringe barrel.

2. Tracheobronchial Washings

A superior method in obtaining bacterial cultures and cytological samples for diagnosis of respiratory disease is by transtracheal aspiration or tracheobronchial wash. Stills *et al.* (1979) reported on a method of transtracheal aspiration in cynomolgus monkeys. The monkeys were restrained in an upright position with the head and neck in complete extension so as to facilitate entry into the trachea. A 17- or 19-gauge intravenous catheter and needle set was pierced through the skin and into the tracheal lumen between the cartilagenous tracheal rings. The needle was withdrawn once the catheter was in a bronchial lumen. To obtain the respiratory fluid sample, 10–15 ml of sterile saline was introduced through the catheter, and then aspirated back immediately into the same syringe. Typically 4 ml of the saline was recovered and could be used for bacterial culture or centrifuged for examination of any cellular sediment.

This tracheobronchial washing technique has been successfully performed in animals which were either generally anesthetized (ketamine) or manually restrained, but with local skin anesthesia. The ketamine anesthesia was regarded as safer for the personnel, but the ability to perform this technique without general anesthesia was considered useful for debilitated animal patients.

Surgical exposure of the trachea may be required for small nonhuman primates with relatively flexible tracheas that lie deep within the cervical musculature. The animal is sedated and placed in dorsal recumbency, and the skin directly above the trachea is prepared aseptically. A surgical incision is made on the ventral midline of the neck, and the paired cervical muscles are retracted laterally to provide exposure of the trachea. The trachea is elevated to a superficial position by the placement of a hemostat along its dorsal aspect. The catheter or needle is inserted directly into the trachea, and the flush and collection are conducted as before (authors' personal experience).

A technique has also been described for tracheobronchial lavage performed via an oral endotracheal tube (Ilievski and Fleischman, 1981). Anesthetized rhesus monkeys were placed in dorsal recumbency, with their heads positioned in complete extension over the table edge. A 3 French, 40.6-cm feeding tube was used as the flush catheter. The outer tube that the catheter was supplied in was modified to serve as an endotracheal tube and to protect the flush catheter from contamination by oropharyngeal flora. The authors used a sterile straight hemostat for stabilization of the epiglottis, instead of a laryngoscope, because of their need to perform the procedure aseptically in multiple animals. For individual animals, a sterile laryngoscope and standard endotracheal tube could be used to facilitate the passage of the flush catheter. In their experience, the authors were able to retrieve 1–2 ml of the initial 5-ml inoculum of saline by rotating the animal from side to side several times and applying gentle pressure to the thorax during aspiration. Fluoroscopy can also be used with this technique to visualize the exact location of the lavage and aspiration (Hannothiaux *et al.*, 1991).

F. Intestinal Contents and Feces

1. Free Catch (Stool Samples)

The simplest method for collecting fecal contents is to obtain a fresh stool sample from the floor of the cage. Rhesus monkeys can be trained to defecate after transfer to a holding cage using food reinforcement (Phillippi-Falkenstein and Clarke, 1992). This manner of fecal sampling is adequate in many cases, such as for intestinal parasite assays and fecal fat or starch estimations. Stool samples are inappropriate, however, for assessing individual disease in group-housed nonhuman primates.

2. Rectal Swabs

Rectal swabs have been found to be superior to stool samples for isolation of *Campylobacter fetus* from marmosets (Kaplan

et al., 1982). The swab was inserted through the anus and rotated around the circumference of the rectal mucosa. It is thought that swabbing of the mucosa increases the recovery of mucosal surface-dwelling bacteria, and thus is superior to simply placing the swab within the anal canal. The disadvantages of rectal swabs are that they require restraint (chemical or manual) of the nonhuman primate patient and that they primarily reflect the status of the colonic mucosa and contents, and not necessarily the characteristics of the small intestine.

3. Intestinal Aspiration

Intestinal aspirates interpret the bacteriological or metabolic characteristics of the small intestine more accurately than stool samples or rectal swabs. However, proper intestinal aspiration requires a surgical approach, general anesthesia, and a sterile technique. Laparotomy permits accurate identification of the intestinal segment which is aspirated, and the sampling of intestinal contents can be coupled with full-thickness biopsy of the associated intestinal wall (see Part B, Section III, D).

4. Intestinal Cannulation/Endoscopy

Flexible fiber-optic endoscopy or sigmoidoscopy is a nonsurgical alternative for localized sampling of gastrointestinal contents. Ford *et al.* (1989) detail the use of a steerable catheter (nonfiber-optic) which was placed in the proximal duodenum via fluoroscopic visualization. The use of these intraluminal catheters to obtain samples of intestinal contents obviates the need for sterile surgery and postsurgical care, but requires time to become accustomed to the equipment and is rather expensive.

G. Bile

The sampling of bile from nonhuman primates is typically a chronic or multiple procedure performed for experimental purposes, although it can be an acute procedure for diagnostic purposes (e.g., cholecystitis). A nonsurgical procedure has been described for acute bile aspiration (Spalton and Clifford, 1979). Surgical collection of bile is the more usual approach, by laparotomy, cholecystopexy, or bile duct cannulation.

1. Duodenal Entubation

A single-lumen Ryle's tube was passed nasogastrically into the duodenum with visual assistance by fluoroscopy. Once properly located in the duodenum, the catheter was flushed with warm water to clear the area of solid intestinal contents, and the fluid contents were then suctioned by vacuum. Pancreozymin and cholecystokinin were injected intravenously to stimulate biliary discharge from the gallbladder, and the bile sample was then aspirated (Spalton and Clifford, 1979).

2. Needle Aspiration

Acute needle aspiration of the gallbladder is easily accomplished via a laparotomy (Glenn and McSherry, 1970). Surgical attachment of the wall of the gallbladder to the inner abdominal wall (cholecystopexy) permits repeated needle aspirations of bile. This technique has been described for the baboon (Thorbjarnarson *et al.*, 1972). The authors commented that removal of the peritoneum and internal rectus fascia at the point of attachment helped to prevent subsequent detachment of the gallbladder. The point of cholecystopexy was marked on the lateral abdominal wall by placement of a circular skin tattoo with a steel suture at the center. Ultrasound guidance of percutaneous needle aspiration of bile from the gallbladder may be possible in some species of nonhuman primates.

3. Bile Duct Cannulation

Chronic cannulation of the bile duct has also been reported as a means of long-term access to bile in nonhuman primates. Techniques for chronic biliary cannulation have been described by McSherry *et al.* (1973), Cloyd *et al.* (1977), and Wood *et al.* (1977). One author (RCD) has had personal experience (1990) with another method of bile duct cannulation, performed in African green monkeys. A 0.065-inch outer diameter silastic catheter was inserted into the distal aspect of the bile duct (but proximal to the pancreatic duct) and directed retrograde toward (but not past) the cystic duct. Connection with a gastrostomy tube maintained enterohepatic circulation.

H. Lymph

The collection of lymph in nonhuman primates is primarily done to retrieve lymphocytes for experimental purposes. Martin and Leiseca (1977) described a surgical technique for thoracic duct cannulation in the rhesus monkey. The duct was cannulated immediately cranial to the cisterna chyli, which traversed the diaphragm in the aortic hiatus. Silastic tubing of outer diameter 0.065 or 0.047 inches was inserted into the cisterna. The other end of the catheter was exteriorized through the body wall, and was left open during collection periods, or knotted and secured to the trunk during rest periods. Recirculating the lymph back into the proximal thoracic duct has been successfully accomplished in thoracic duct cannulations of African green monkeys (authors' personal experience, 1990).

I. Peritoneal Effusions

The collection of peritoneal fluid is important for the differential diagnosis of ascites-producing diseases, such as congestive heart failure, peritoneal neoplasia, or peritonitis. Peritoneal fluid collection is also performed for experimental purposes, such as collection of stimulated macrophages. It is important to

maintain aseptic conditions when tapping the peritoneum in order to prevent contamination of the fluid sample as well as the peritoneal cavity itself.

Heisey and Sankaran (1981) studied four methods of harvesting peritoneal fluid from cynomolgus monkeys for collecting peritoneal macrophages. The most successful technique utilized a 13-gauge cannula with a 17-gauge needle inserted into the cranial aspect of the right abdominal wall. The tip of the cannula was modified such that it had 10–15 perforations made by an 18-gauge needle. After 100 ml of harvest medium was introduced, the abdomen was lightly tapped on both sides to distribute the medium, and the fluid was removed by gentle aspiration. This method was superior to using an 18-gauge needle only, which repeatedly became occluded with omentum, or abdominal wall penetration with a 6-mm biopsy needle, which resulted in contamination of the collection with erythrocytes.

J. Pleural Effusions

The collection of abnormal thoracic fluids is essential for diagnosis of the cause for their accumulation, as well as to relieve any respiratory distress that may be caused by the presence of large volumes of fluid in the pleural cavity. It is generally recommended that thoracic radiographs be taken to identify the distribution of the fluid and that the animal be positioned so that the fluid accumulates dependently in a suitable location. The fluid can be tapped with either a standard intravascular catheter or a Butterfly infusion set attached to a three-way stopcock and a large volume (greater than 12 ml) syringe. The bevel of the needle should be directed toward the parietal pleura so as to prevent the lungs from obstructing the lumen (Richardson and Janas, 1985). A larger bore "chest tube" with multiple fenestrations may need to be implanted surgically to permit ready withdrawal of large volumes of accumulated fluid.

K. Semen/Sperm Collection

As with most animal species, electroejaculation is the primary means by which to obtain semen or sperm samples from nonhuman primate males. The successful collection of ejaculate from macaques using direct penile electrostimulation was initially described by Mastroianni and Manson (1963) and was further assessed by Valerio *et al.* (1969) and Settlage and Hendrickx (1974). Sarason *et al.* (1991) evaluated the capability of nonmetal electrodes (modified defibrillation pads) for direct penile electrostimulation in macaques; this alteration removed the potential hazard of superficial burns on the penile epithelium.

The use of rectal probes for ejaculate collection has been described for a variety of species of nonhuman primates (Fussell *et al.*, 1967; Gould *et al.*, 1978) and also specifically for squirrel monkeys (Bennett, 1967; Lang, 1967), macaques (Weisbroth and Young, 1965; Gilman, 1969), baboons (Bornman *et al.*, 1988), and great apes (Warner *et al.*, 1974). Comparison studies of direct penile and rectal probe electroejaculation techniques in macaques have been performed by Van Pelt and Keyser (1970), Matsubayashi (1982), and Gould and Mann (1988). In all three studies, the total sperm count was significantly greater by the direct penile stimulation method. Comparisons between these studies and or other technique reports must be made carefully, as many aspects of the experimental design (e.g., type of restraint, probe design, and stimulus voltage, duration, and interval) vary between investigations.

Other techniques used occasionally to obtain semen samples are the use of an artificial vagina (Fussell *et al.*, 1973) and collection of masturbatory ejaculate. Gould (1990) reviewed techniques for ejaculate collection in great apes. Rectal probe stimulation was the most common method utilized, as the apes could be totally anesthetized, in contrast to direct penile electrostimulation or the use of an artificial vagina. Rectal probe stimulation was reported to be enhanced by prostatic massage. Masturbated samples were considered to be the least desirable, as alteration by the environment and depletion of the liquid fraction were readily possible.

L. Uterine Fluid/Ova Collection

Nonsurgical uterine flushing has been performed in a wide variety of animal species for both oocyte collection and embryo recovery. Pope *et al.* (1980) collected oocytes from baboons using an Isaac's endometrial cell sampler which was modified to have a double-lumen catheter. This enabled a continuous flow of warm culture medium (10 ml total, instilled at a rate of 1–2 ml/min) into (and out of) the uterus. The success rate for collection of eggs or embryos approached 50%. This technique was modified for rhesus monkeys by Goodeaux *et al.* (1990). Cannulation of the uterus is difficult in macaques because of the tortuous cervix. This was overcome by using a tapered 17-gauge trochar to advance the cannula past the cervix and by assisting with its passage via palpation per rectum. Bavister *et al.* (1985) also describe a technique for cervical cannulation in rhesus monkeys using a blunt 17- or 18-gauge needle with an internal wire trochar. Ariga and Dukelow (1977) reported on a method of uterine flushing in the squirrel monkey which used laparoscopy to visualize the uterus for percutaneous injection of warmed media into the lumen. Fluid and oocytes were collected by withdrawal via a catheter inserted through the cervical canal. Kraemer *et al.* (1983) tried several versions of a double catheter system for embryo collection in the baboon; the success rate after 26 attempts with the various catheters was 19%, and cervical or uterine irritation occurred with the use of some catheter styles.

Surgical methods for embryo or oocyte collection in nonhuman primates in which a laparotomy is used to visualize and manipulate the reproductive tract have also been described (Hendrickx and Kraemer, 1971; Kraemer *et al.*, 1979, 1983). The basic technique involves the instillation of culture medium

through a needle inserted directly into the lumen of the uterus or uterine tubes, with collection occurring at the oviducts (either cannulated or free-caught after retrograde flush), by a second needle positioned within the uterine lumen, or by flow through the cervix and out the vaginal canal.

Oocytes can also be obtained directly from the ovarian follicles using either laparoscopy or laparotomy. Direct follicular aspiration during laparoscopy has been described for the rhesus monkey (Bavister *et al.*, 1983) and for the great apes (Gould, 1983, 1990). Laparoscopic follicular aspiration is currently the method of choice in rhesus monkeys (Bavister and Boatman, 1993; Wolf and Stouffer, 1993) and squirrel monkeys (Dukelow, 1993). The technique that Bavister and associates have used is vacuum-assisted aspiration through a Teflon-protected (lined) stainless-steel needle. This catheter system was first designed by Renou *et al.* (1981) for use in collecting human oocytes; Bavister's modification consists of an additional 21-gauge needle attached to the tip to recover oocytes from the smaller follicles in macaques. VandeVoort and Tarantal (1991) utilized ultrasound to visualize mature ovarian follicles in cynomolgus monkeys following hormonal stimulation and to guide the percutaneous insertion of a 22-gauge 3-inch spinal needle for aspiration of oocytes.

M. Amniotic Fluid

Amniocentesis has been performed in nonhuman primates for animal model assessment of the procedure as done in humans and also for veterinary diagnostic procedures. Poswillo (1972) used nine cynomolgus monkeys at Days 39–47 of gestation to evaluate amniocentesis in the first trimester of pregnancy. Following exposure by laparotomy, a 17-gauge needle was advanced blindly toward the center of the uterus until clear fluid was obtained. Between 1.0 and 1.5 ml of amniotic fluid was collected and nine healthy fetuses were delivered without complication by cesarean section at term. Hislop *et al.* (1984) used cynomolgus monkeys to demonstrate that amniocentesis early in the second trimester (57–89 days) could lead to structural and functional pulmonary abnormalities. Varying amounts of amniotic fluid were removed, ranging from 0 (technique only) to 57.5 ml.

Percutaneous transabdominal amniocentesis in the near term (>120 days) rhesus monkey was described by Epstein and Chez (1976). The presenting portion of the fetus was pressed anteriorly, and a 22-gauge, 4-inch spinal needle (without stylet) was inserted into the lower uterine segment. The insertion point was on the midline, 3 cm cranial to the pubis, and at a 45° angle. Only 3 of 30 monkeys had reduced amniotic fluid volumes at the time of cesarean delivery. Hess *et al.* (1979) used a similar technique in 80- to 120-day gestation rhesus monkeys to obtain amniotic fluid samples for antenatal sex determination. They inserted a shorter (1–1.5 inch) 22- or 23-gauge needle and attached the collection syringe only after amniotic fluid flowed from the needle hub. This procedure did not result in any pregnancy complications. Tarantal (1990) used ultrasound to identify the amniotic sac, and obtained small amounts (approximately 1.0 ml) of amniotic fluid from earlier in gestation (beginning at Day 60).

III. TISSUE COLLECTION

A. Skin

1. Skin Scraping

Skin scrapings are typically performed for diagnosis of ectoparasite infestations. Mineral oil is placed on a glass slide, and a scalpel blade is dipped in the mineral oil and scraped across the skin at the site of a typical lesion. The scraping should be deep enough so as to cause some bleeding; this is necessary to recover those species of mites which burrow into the epidermis.

2. Punch Biopsy

Biopsy of the skin is a common procedure for the diagnosis of dermal lesions. It is critical that both affected and normal tissue be obtained during the biopsy so that accurate interpretations can be made with regard to the status of the lesions. Recently formed, fully developed lesions that are typical for the condition should be sampled, and traumatized, encrusted, or treated lesions should be avoided. Surgical preparation should be performed if the sample is to be submitted for bacterial isolation, otherwise only a minimal alcohol prep should be done to minimize ultrastructural damage due to vigorous scrubbing (Richardson and Janas, 1985).

Punch biopsies are simple to perform and can be done under local anesthesia, although sedation may be necessary for restraint. A variety of skin biopsy punches are available, typically in diameters of 4–8 mm. A Walsh pressure ring may be used to stabilize the skin and to control local bleeding. Rotational motion on the handle of the punch and slight downward pressure are used to cut through the dermis to the subcutaneous layer. The circular skin sample is severed from its subcutaneous attachments and is prepared as necessary for analysis; sutures may be needed to close the defect.

3. Excisional Biopsy

Larger, full-thickness biopsies can be obtained by surgically excising the affected portion of skin. Excisional biopsies are especially indicated when an entire focal lesion can be removed without complication. To diagnose psoriasis vulgaris in a cynomolgus monkey, elliptical pieces of lesioned and unaffected skin were removed with a scalpel blade and placed on a tongue depressor, dermis side down, for formalin fixation (Jayo *et al.*, 1988). Personal communication with the primary author revealed that larger excisional biopsies were necessary for their

diagnostic endeavors; punch biopsies were not contributory. It is recommended that electrocautery not be used in obtaining skin biopsies because of the histologic damage caused by the high temperatures.

B. Liver

Biopsy of the liver is a common technique for the diagnosis of hepatic disorders as well as for experimental sampling purposes. Liver biopsies are either "open" or "closed," based on whether or not the abdominal cavity is opened and exposed. The advantages of closed biopsy techniques are that they are rapid, do not constitute major survival surgery, and usually require only mild chemical restraint. The advantages of open procedures are that they permit visualization of the viscera for sample site identification and assurance of hemostasis and permit the acquisition of larger samples.

1. Percutaneous Needle Biopsy

Closed needle biopsy techniques for liver tissue have been reported for nonhuman primates. Miller *et al.* (1978) described percutaneous liver biopsies in rhesus monkeys using a 1.9 × 70-mm biopsy needle. The needle was inserted into the right side at the point of maximum dullness (by percussion) between the seventh and ninth ribs at the midaxillary line. A trochar was used to penetrate the skin, and the needle was guided through the intercostal muscles into the peritoneal cavity. The monkey was rotated to a dorsally recumbent position, and saline was flushed through the biopsy needle to clear it of any muscle or connective tissue. The needle was then inserted into the liver at right angles to the liver surface and body wall, and suction was applied to the syringe after insertion to aid in recovering biopsy tissue. Three punches were made per needle, and the sample size averaged 83 mg.

Voss (1970) performed closed liver biopsies in tamarins and marmosets using a 16-gauge Klatskin needle, which permitted aspiration of the biopsy sample into the syringe for visualization. The animals were maintained in a dorsally recumbent position or were restrained in a vertical position, which was preferred for sampling livers of normal size since the liver descended to a more accessible location below the ribcage. The sampling procedure itself was similar to the technique of Miller, except that the needle was inserted at a 45° angle to the ventral body wall 1.0 cm posterior to the xiphoid process. In studies of viral hepatitis requiring hepatic biopsies in chimpanzees, Menghini needles (Popper *et al.*, 1980) and Vim-Silverman needles (Schaff *et al.*, 1984) have been used.

2. Ligature or Finger Fracture

The ligature or finger fracture techniques of liver biopsy are open techniques which permit sampling of large pieces of liver (1–10 g). The liver lobe is bisected at the designated point with the surgeon's fingers, large-blade (Carmalt) forceps, or a piece of sterile suture material. The piece to be sampled thus remains attached to the remainder of the liver by the larger blood vessels and bile ducts, permitting their ligation for hemostasis. Once the vessels are secured, the tissue portion is removed completely, and the sinusoidal hemorrhage is controlled with gelatin hemostat (Gelfoam, Upjohn, Kalamazoo, MI). The ligature fracture technique is very successful in cynomolgus and African green monkeys, and can be used for multiple samplings from different lobes in an individual animal (Talcott and Dysko, 1991).

3. Punch Biopsy

Punch biopsy of the liver is essentially identical to the technique used for punch biopsy of the skin, and in fact uses the same instruments. The punch is twisted into the hepatic parenchyma and is rotated to remove a partial thickness sample. Gelatin hemostat or omentum is placed into the defect to control any subsequent hemorrhage (Boothe, 1990).

4. Wedge Biopsy

Some wedge biopsies simply involve the removal of a desired piece of liver and packing the incised defect with a gelatin sponge (Voss, 1970). Although this technique may be successful occasionally, it does not ensure adequate hemostasis. Nolan and Conti (1980) used an automatic stapling device to obtain 10- to 20-g liver tissue specimens from chimpanzees. The device placed the staples in a staggered double row at a point of compression, thus assisting in the control of hemorrhage once the free edge of the liver was dissected away. A technique using horizontal mattress sutures instead of staples was described by Eichberg (1985). In this case the sutures were placed in a double overlapping row, and the liver was severed gradually as the sutures were tied. This technique permitted sample sizes of 50–100 g from chimpanzees.

C. Kidney

1. Percutaneous Renal Biopsy

Closed renal biopsies possess the same advantages and disadvantages as closed liver biopsies. Accurate location of the kidney is important, which can be done by manual palpation and stabilization in the smaller nonhuman primates or by prebiopsy intravenous pyelograms in larger primates. Moser *et al.* (1967) described a technique for percutaneous renal biopsy in the chimpanzee. After several intravenous pyelograms, the authors found that the left kidney was relatively fixed in its relationship to the iliac crest and the last rib. Thus those bony landmarks were used to introduce the biopsy needle into the abdominal cavity. Vim–Silverman–Franklin and Vim–Silver-

man biopsy needles (20 and 13 cm long, respectively) were inserted at right angles to the back to obtain successful samples. In macaques, the right kidney is usually fixed in position such that the caudal pole is palpable just beyond the end of the ribcage. This permits external manual stabilization of the kidney to assist in location and successful passage of the biopsy needle (authors' personal experience). The Tru-Cut biopsy needle (Travenol Laboratories, Deerfield, IL) is a commonly used instrument for percutaneous renal biopsies in companion animals, and would probably work quite well for biopsies from a variety of organs in nonhuman primates. It features an inner obturator-specimen rod, which has a specimen notch cut into its proximal end, and an outer cannula, which is advanced over the specimen notch to cut the desired tissue free from the rest of the organ. Spring-loaded biopsy guns (e.g., Bard Monopty Instrument, C. R. Bard, Inc., Covington, GA), which mechanize the process by which the outer cannula is advanced over the obturator-specimen rod to cut the biopsy sample, could also be used for renal biopsies in nonhuman primates.

2. Open Biopsy Techniques

Needle biopsy is also a common method for sampling renal tissue during laparotomy procedures. The needle is inserted near the caudal pole of the kidney and is directed craniad (Boothe, 1990). Wedge biopsies of the kidney provide larger samples for analysis and can guarantee sections of the corticomedullary junction, but carry a greater risk of intra- and postoperative hemorrhage. Typically a scalpel is used to incise the renal capsule and remove a wedge-shaped piece of cortex and medulla. Mattress sutures are used to close the defect, and omentum can be incorporated in the closure to help provide hemostasis (Boothe, 1990).

D. Gastrointestinal Tract

1. Endoscopy/Colonoscopy

Fiber-optic endoscopy and/or colonoscopy is a proven method in obtaining intestinal mucosal biopsies. Endoscopy permits visualization of the intestinal mucosa to be sampled, does not always require general anesthesia, and does not constitute major survival surgery. Care must be taken, however, so as to not perforate the intestinal wall with a full-thickness biopsy. Another disadvantage of endoscopy is that endoscopes cannot enter the jejunum or ileum easily, and so only the colon, stomach, and duodenum can be biopsied in this way. Clapp *et al.* (1987) described a colonoscopic technique for mucosal biopsy in marmosets and tamarins. A 4.8-mm fiber-optic pediatric bronchoscope was used to visualize the mucosa, and biopsy forceps were passed through the channel on the bronchoscope to obtain the mucosal sample. The authors were able to obtain samples from the ascending colon in the marmoset, a distance of 20–25 cm from the anal sphincter. The authors emphasized the need to visualize carefully the jaws of the biopsy forceps as they obtained the tissue from the colon; this was crucial in reducing the incidence of intestinal perforation.

General recommendations for colonoscopic biopsy include fasting the animal for 24–36 hr and administering warm water enemas at 18 and 2 hr preoperatively. Observation of the mucosa of the large intestine is aided by air insufflation to dilate the lumen (Richardson and Janas, 1985).

2. Laparotomy

Excisional biopsy of portions of the gastrointestinal tract is advantageous when full-thickness tissue samples are required or when samples are needed from portions of the tract inaccessible via endoscopy (i.e., jejunum and ileum). Intestinal biopsy following laparotomy is also preferred when samples of the intestinal contents are warranted (e.g., for microbiological culture) in conjunction with the tissue sample. Boothe (1990) prefers to make biopsy incisions transverse to the mesentery, and recommends that the sample not exceed 20% of the intestinal circumference. Closure of the defect is with a single layer of synthetic absorbable or monofilament nonabsorbable suture in a simple interrupted pattern. Coverage of the biopsy site with the greater omentum is recommended to protect the wound. Longitudinal incisions for intestinal biopsy can also be closed as if they were a transverse incision in order to prevent postoperative intestinal stricture (authors' personal experience, 1983).

E. Spleen

Percutaneous biopsy of the spleen is possible, as long as the spleen can be palpated adequately through the abdominal wall. Lipowitz *et al.* (1985) recommended fine needle biopsy with a 25-gauge needle for splenic biopsy in companion animals, although other percutaneous biopsy instruments could be used.

Biopsy during laparotomy can be performed using any of the needle aspiration or punch biopsy techniques typically performed percutaneously. Incisional biopsy can be obtained readily from the splenic margin by placing full-thickness mattress sutures across the width of the spleen and transecting the biopsy sample peripheral to the sutures. Successful biopsy of the spleen has been accomplished in marmosets and tamarins by simple removal of a wedge-shaped piece of splenic tissue during laparatomy and replacing the defect with gelatin hemostat (Voss, 1970). Total splenectomy is typically indicated for involved splenic lesions; this should be considered carefully in feral nonhuman primates because of the increased possibility of latent hemoparasitism (e.g., malaria and hemobartonella).

F. Lymph Nodes

Lymph node biopsies are typically performed for diagnosis of lymph node enlargement. All of the percutaneous biopsy

techniques noted for other parenchymatous organs, such as needle aspiration or Tru-cut needle biopsy, are possible for peripheral lymph nodes. These techniques can also be performed on internal nodes in association with exploratory surgery. A "guillotine biopsy" can also be performed after surgical exposure of a node (Jeglum and Dulisch, 1985). This technique involves looping absorbable suture material around the circumference of a node and tightening it to compress the node into an "hourglass" shape. One end of the node is then removed with a scalpel blade. Wedge biopsies and total excision of the node are also commonly used to obtain lymph node samples for analysis. Excisional biopsies are recommended for mesenteric and iliac lymph nodes when chosen for sampling during laparotomy (Boothe, 1990).

G. Lung

1. Percutaneous Needle Biopsy

Richardson and Janas (1985) recommend the Lee biopsy needle (Unique Industries, Memphis, TN) for percutaneous lung biopsy. This instrument has a specimen notch and a narrow-gauge lumen which allows for aspiration while cutting. Radiography, preferably fluoroscopy, is recommended to help determine the approach for the biopsy. Possible complications from percutaneous thoracic biopsies include pneumothorax and hemorrhage, manifested as either hemothorax or hemoptysis.

2. Bronchoscopy/Thoroscopy

Bronchoscopy can be used to obtain tissue samples from intraluminal tracheal or bronchial masses, or even from peribronchial lung parenchyma. Thoroscopy can be performed with either a rigid or a flexible endoscope, permitting visualization of the pleural surfaces of the lungs, pericardium, and thoracic wall. Complications with bronchoscopy and thoroscopy are identical to those associated with percutaneous lung biopsy.

3. Partial and Total Lobectomy

Partial or total lung lobectomy is preferred for most instances warranting lung biopsy as they permit direct visualization of intrathoracic organs and lesions, which can be sampled adequately without the complications inherent in blind, nonsurgical approaches. The disadvantages of these techniques are that they are expensive and time-consuming, constitute major survival surgery, and require general anesthesia, full surgical preparation, and positive pressure ventilation. Nelson (1985) described several techniques for lobectomies in companion animals; these can be easily adapted for use in nonhuman primates.

H. Bone Marrow

As with some of the parenchymatous organs, bone marrow samples can be obtained either by needle aspiration or by punch (core) biopsy. The preferred sites for sampling the bone marrow include the iliac crest, the greater trochanter of the femur, the greater tubercle of the proximal humerus, and the tibial tuberosity. Other sites have been used, including the sternum, the marrow of the rib, and the ischial tuberosity of the pelvis (Wisecup *et al.*, 1968). The advantages of the iliac crest are that it is readily accessible and it is a fairly active site of marrow production (Perman *et al.*, 1974). The iliac crest is not recommended for aspiration or biopsy in small dogs or cats because of the small size of the marrow space; this should also be considered when selecting biopsy locations in the Callitrichids and other small nonhuman primates. Most of the techniques discussed next are from three references on bone marrow biopsy in the dog and cat (Perman *et al.*, 1974; Richardson and Janas, 1985; Cotter and Blue, 1985).

1. Needle Aspiration

Bone marrow aspirates from the ischial tuberosity were used to determine marrow cellularity and architecture in anemic chimpanzees (Wisecup *et al.*, 1968). One to 2 ml of marrow was obtained per aspirate using a 2-cm long, 14-gauge needle. Several different styles of bone marrow aspiration needles are available (e.g., Rosenthal, Osgood); these needles vary in their length, gauge (typically 13–18), bevel, and handle style. The specific needle selected for aspiration depends on the size of the animal patient and the biopsy site chosen. Bone marrow aspirates of the iliac crest can be made from either the dorsocranial or the lateral aspects. The dorsocranial approach can be performed with the animal in lateral or sternal recumbency. The needle is seated into the bone at the widest part of the dorsal ilium and is inserted into the marrow cavity parallel to the two sides of the ilial wing. The lateral approach to the ilial marrow is a point approximately 1 cm ventral to the site noted for the dorsocranial approach. The advantage of the lateral approach is that the needle seats better into the flat side of the ilium; the disadvantage is that the marrow cavity is entered at its narrowest dimension, and care must be taken so as not to penetrate the medial cortical surface. The point of entry for aspiration from the greater trochanter of the femur is actually immediately medial to the trochanter and parallel to the femoral shaft.

2. Core Biopsy

A core biopsy of bone marrow provides information on the architecture of the marrow as well as the cellularity, and is necessary for the diagnosis of conditions which cause "dry" aspirates, such as aplastic anemia or myelofibrosis. Core biopsies are also more diagnostic for patchy, multifocal lesions, such as neoplastic metastases or tuberculous granulomas (Cotter and Blue, 1985). The Jamshidi needle is the preferred instrument for obtaining core samples; it can also be used for aspirates. The needle is conical and is beveled specifically to prevent the bone

from lodging in the tip. The sites and procedures for obtaining core samples are essentially the same as for the needle aspirate.

I. Bone

1. Iliac Crest

The iliac crest is a preferred site for obtaining bone biopsy samples in nonhuman primates because it is composed of both cortical and cancellous bone, permits easy surgical access with minimal postsurgical discomfort, and allows for subsequent contralateral biopsy for serial evaluations. Goodwin and Jerome (1987) successfully performed iliac crest biopsies in both baboons and cynomolgus monkeys. Following general anesthesia and aseptic preparation of the surgical site, the nonhuman primate patient was placed in sternal recumbency. A skin incision was made from the craniodorsal iliac prominence to a point along the crest 8 cm caudal. All underlying musculature was incised down to the fascia and periosteum, which were incised and reflected. Two parallel biopsy cuts were made with an oscillating saw; the cuts were 1 cm deep, 1 cm apart, and the cranial cut was 1–1.5 cm caudal to the iliac prominence. The sample was readily removed with a light tap on a bone chisel. Periosteal and fascial layers were reapposed, as were the muscular and subcutaneous layers and skin edges. This technique provided a quality specimen of cortical and cancellous bone for analysis. Inskip *et al.* (1992) modified this technique slightly in a study requiring skeletal lead analysis in cynomolgus monkeys. The parallel cranial cut was replaced by a perpendicular cut which transected the ilium and removed the cranial iliac prominence.

Nogues and Milhaud (1988) obtained iliac crest samples from several rhesus monkeys. The general surgical approach was the same as previously described, except that bone-cutting pliers were used to take a triangular tissue sample from the top of the iliac crest, at the iliac tuberosity. This sample was 1.0–1.2 cm at the base and approximately 1.5 cm deep.

Klein *et al.* (1991) used an 8-mm internal diameter Michele bone trephine (V. Mueller Co., Chicago, IL) to obtain transilial core biopsies from baboons. The site location (3–5 mm ventral and cranial to the craniodorsal aspect of the iliac crest) was chosen based on the presence of adequate trabecular bone and parallel cortical surfaces for histomorphometric analysis.

2. Vertebral Body

Percutaneous biopsy of the vertebral body has been performed successfully in rhesus monkeys for a study investigating changes to the cancellous bone of the weight-bearing axial skeleton (Hermann and Smith, 1985). Two techniques were used: a freehand method which required the placement of external landmarks (needles) and multiple radiographs, and a method which used a pneumatic trephine guide set according to a single radiograph and trigonometric calculations. Both techniques utilized a 3-mm-diameter trephine which obtained samples approximately 2 cm long.

3. Bone Lesions

Bony lesions in various locations may need to be biopsied for definitive diagnosis. Richardson and Janas (1985) recommended the use of the Michele bone trephine to obtain bone biopsy samples in companion animals. The biopsy site is determined by radiography, and the overlying skin and musculature are incised to permit direct contact of the trephine to the bone. The trephine is rotated into the bone to an appropriate depth, and the biopsy sample is broken free by rocking the trephine back and forth. The biopsy defect is packed with bone wax or gelatin hemostat to prevent postsurgical hemorrhage. Power-driven trephines are not recommended because the heat from the instrument can damage the biopsy sample.

J. Muscle

1. Skeletal Muscle

Skeletal muscle biopsy biopsy specimens can be obtained readily from superficial muscles through small incisions in the overlying skin and fascia. Howard (1975) chose the vastus lateralis muscle of the thigh from which to obtain biopsy samples in *Macaca nigra*; Moolenbeek and van Knapen (1981) selected the biceps muscle of the upper arm in cynomolgus monkeys. These authors performed serial muscle biopsies and reported that repeat sampling too close to previous biopsy site scar tissue led to hemorrhage and suture dehiscence. They eventually utilized other superficial skeletal muscles, such as the triceps of the upper arm and the quadriceps group of the thigh, and emphasized that incising longitudinally in the direction of the muscle fibers greatly facilitated a successful outcome.

2. Endomyocardium

The increased incidence of endomyocardial biopsy in man and laboratory animals is directly related to the continued success of cardiac transplantation and the need to study and monitor the rejection process. Rose *et al.* (1986) conducted a study on the histologic appearance of multiple endomyocardial biopsies in baboons, using the technique described by Cooper *et al.* (1982). A 9 French Kifa catheter was inserted into the jugular vein by a percutaneous approach and was stabilized in the right ventricle. The bioptome was then inserted into the catheter, and endomyocardial biopsy samples were obtained from the right ventricular apex, using fluoroscopic control. Samples were typically in one to four pieces, each approximately 1–2 mm in diameter.

K. Uterus

Samples of the uterine wall and endometrial lining are typically taken by wedge biopsy following laparotomy. Eley *et al.* (1991) used a punch biopsy technique (1.7 mm internal diameter) to obtain endometrium from baboons for microscopic evaluation. Laparoscopic examination and biopsy of the uterine wall for endometriosis in baboons have also been described (Cornillie *et al.*, 1992). Olson and Sternfeld (1987) described a percutaneous uterine biopsy technique which was successful in rhesus monkeys with enlarged uteri. The uterus was manually pressed against the lateral abdominal wall by placement of a finger or probe within the rectum of the patient. A TruCut biopsy needle was inserted into the abdominal cavity via a small skin incision and was then inserted into the uterus to obtain a full-thickness biopsy of approximately 2 × 10 mm. This technique was not successful in obtaining cervical biopsy samples. The authors admitted that a biopsy technique which permits visualization of the sample site should be used for small or soft uteri.

L. Testicles

Percutaneous needle biopsy techniques used for other parenchymatous organs are suitable for testicular biopsy. Testicular aspiration has also been utilized to obtain cells for DNA flow cytometry. Hellström and Kaack (1990) evaluated testicular aspiration and biopsy techniques in the baboon with regard to cellular quality and consistency of flow cytometric results. The biopsy was performed using a Biopty gun (Bard Urological, Covington, GA) with a 20-gauge 16-cm Biopty needle. There was no significant difference in cellular distributions between biopsy samples and aspirates taken with a 3.8-cm Westcott needle, but more cellular debris (primarily skin and tunica albugenea) was present in biopsy samples.

IV. THERAPY ADMINISTRATION

Potential stresses placed on a nonhuman primate patient need to be considered prior to the administration of therapeutic agents. Treatments that do not require capture, such as oral administration via the food or water supply, should be given proper consideration. Longer acting substances, such as timed-release or once-a-day treatments, should also be considered for nonhuman primates. This is especially true of group-housed or free-ranging animals, in which capture and administration can be stressful to both the patient and its conspecifics.

A. Intravenous

Much of what is described for blood collection techniques is applicable for intravenous therapy administration (see Part B, Section II, A). Typically the lateral saphenous vein is the best choice for the instillation of needles or catheters. Angiocaths (The Deseret Company, Sandy, UT) can be maintained for several days in nonhuman primates, but precautions must be taken to ensure that the patient cannot readily remove the catheter upon recovery from anesthesia. The use of lightweight fiberglass casting tape (Lightcast II, Merck Sharp & Dohme Orthopedics, Costa Mesa, CA) was described by Conti *et al.* (1979) as a means of protecting and immobilizing a saphenous catheter for a 2-week duration in nonhuman primates. Infusion ports and pumps which can be implanted subcutaneously (Infusaid Corporation, Norwood, MA) have been used successfully in nonhuman primates for the chronic intravenous delivery of therapeutic and experimental agents (Schmutzler *et al.*, 1988). Severely debilitated animals can often be catheterized without difficulty, but the potential for improvement in health status warrants measures to secure the catheter, especially if recovery can be dramatic (e.g., response to hypoglycemia therapy).

B. Intramuscular

Intramuscular administration of therapeutic agents is often preferred for nonhuman primates since such injections can be given readily to animals housed in restraint-back cages or by pole syringe to group-housed animals. As with most animal species the preferred location is in the larger muscles of the caudal thigh, with the cranial quadriceps group, the gluteal muscles, and the caudal head of the triceps on the arm as alternate choices.

C. Subcutaneous

Subcutaneous administration of therapeutic agents is also possible in animals maintained in restraint-back cages, although accuracy in delivery of the substance to the subcutaneum instead of intradermally or intramuscularly is slightly more difficult to ensure. The most common use of the subcutaneous route is to deliver replacement fluids in instances when intravenous administration is not critical or practical. These fluids should be isotonic to plasma and contain no dextrose so that their subcutaneous administration will not cause osmotic extravasation of intravascular fluid. Hyaluronidase (Wydase, Wyeth Laboratories, Philadelphia, PA) can be added to fluids to be delivered subcutaneously to cause temporary dissolution of the hyaluronic acid connections in this connective tissue space. This permits the fluids to disperse evenly through the subcutaneous space instead of accumulating in the classic large pressurized blebs.

D. Oral

Several different methods have been used to administer medications orally to nonhuman primates. The least stressful

method to provide substances *per os* is by masking the substance in the food or water, although the success of this method is dependent on the appetite of the patient as well as its individual taste preferences. It may take several attempts to find a mixture that the animal accepts readily and thus ingests the medication completely. Typical approaches include injecting the substance into pieces of fruit, strained baby food, or sugar cubes (Whitney and Wickings, 1987). Preconditioning nonhuman primates to accept an oral treat (e.g., juice, bread, and jam) daily would assist with the need for occasional oral therapy as the animals would be conditioned to accepting a carrier for the medication. For large-scale dosing of medications, commercial monkey chow could be ground, dosed, and reformed or special diets could be created from individual ingredients and include the medication dosed on a per calorie basis.

The drinking water can also be medicated using an individual water bottle. Automatic watering systems should be disconnected, and the water should be flavored or sweetened with sugar or commercial drink mixes to improve palatability. Oral electrolyte solutions can often be supplied to nonhuman primates in individual bottles to help maintain proper fluid/electrolyte balance in animals with modest cases of diarrhea (authors' personal experience, 1987).

In instances when oral administration is necessary, but cannot be accomplished through volunteerism of the patient, direct oral dosing is possible. Direct dosing is especially important in anorexic animals which require tube feeding to provide nutrients or in experimental or therapeutic instances in which a precise dose must be given. Oral dosing of marmosets can be accomplished using a metal feeding tube, such as that used to administer substances orally to rodents (Hearn, 1987). For squirrel monkeys and Old World primates, orogastric or nasogastric intubation is recommended (Dukelow and Asakawa, 1987; Whitney and Wickings, 1987). Typically the animals are manually restrained or lightly sedated, and a small (8–12 French) pediatric feeding tube is passed through the nares or an oral speculum into the esophagus and stomach. Care must be taken to ensure that the catheter does not enter the trachea and that the amount of substance administered does not greatly dilate the stomach. Five milliliters is recommended for administration to the squirrel monkey (Dukelow and Asakawa, 1987); 150 ml is the recommended maximum for a 6-kg macaque (authors' personal experience, 1986). Substances should be infused into the stomach as a slow, steady bolus over several minutes.

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PART C. RESTRAINT

Hilton J. Klein and Kathleen A. Murray

I. INTRODUCTION

The use of nonhuman primates in biomedical research has proven extremely beneficial in contributing to medical and surgical advances for the diagnosis, treatment, and cure of many disease conditions affecting both humans and animals. Nonhuman primates are often selected for use in different animal model development or testing regimens because their anatomical, physiologic, and metabolic characteristics are similar to humans (Taylor, 1972). Because of their close evolutionary relationship to humans, large numbers and diverse species of New World and Old World nonhuman primates are used in modern biomedical research laboratories. Unlike other laboratory animals commonly used in research, their use in the biological research laboratory requires special considerations regarding their care, their housing, and, more specifically, their handling and restraint. Handling and restraint procedures are required for performance of physical examinations, diagnostic exercises, dosing, collection of tissue or body fluid samples (e.g., blood, urine, saliva or tears), and during routine colony health and maintenance procedures (e.g., cage cleaning or transportation) (Clifford, 1971; Whitney *et al.*, 1967, 1973).

Because of their size, strength, agility, intelligence, and anatomical variations, the problems encountered in handling and restraining nonhuman primate species are manifold. The use of proper restraint devices and techniques allows safe handling of the animal while minimizing stress and alterations in physiologic parameters in the animal itself and others in the colony (Berendt, 1968; Turkkan *et al.*, 1989). Additionally, any restraint technique or device must be used in a manner that employs good safety practices for animal care or laboratory personnel so that the chances of injury or disease transmission are minimized (Klein, 1989; Lairmore *et al.*, 1989; Muchmore, 1987; Rendquist, 1987; Richardson, 1987). Individuals utilizing nonhuman primates in research should be thoroughly familiar with behavioral aspects that are peculiar to each species before they are used in experimental procedures which require their handling and restraint.

Several general factors need to be considered before a restraint method or device is selected. The most important factor is the species of nonhuman primate used in the research protocol. Considerable variation exists among species of nonhuman primates regarding their size, strength, temperament, and anatomy. These factors and others determine the method selected for effective and safe handling and restraint. Other factors to consider may include the age, sex, health, and reproductive status of the animal. Investigators using nonhuman primates should consider the housing situations involved as well as the purpose and duration of the restraint expected (Fleischman and Chez, 1974; Golub and Anderson, 1986; Myers *et al.*, 1988). Experimental design and the use of hazardous agents (e.g., radioisotopes, infectious agents) are critical factors in selecting the appropriate restraint.

Full consideration must be given to any special circumstances when deciding the appropriate method of restraint. For example, some female nonhuman primates, such as the baboon, exhibit significant swelling of the sex skin at certain stages in their estrus cycle so physical restraint in a chair at this stage may cause injury. The use of padding or cushioned material or consideration of an alternate method of restraint would be appropriate. Another consideration would include selecting a restraint or handling method that minimizes disturbances of the social structure and relationships in a colony when an animal must be removed from group housing (Reinhardt *et al.*, 1990; Salzen, 1989). The difficulties will vary with the hierarchal status of the nonhuman primate removed and the duration of removal if return to the group is desired. If the dominant animal is removed, subordinates may fight to regain the dominant position. When the animal is returned to the colony, fighting may occur because of a change in relationships. In general, some species of nonhuman primate such as the macaques can be successfully reintroduced into the group if the removal period has been less than 3 days (Pare and Glavin, 1986). Individuals responsible for maintaining group-housed primates should be thoroughly familiar with species-specific social behavior when they remove them for the colony handling and restraint procedures.

A final example of relevant, special considerations involves the restraint of sick animals or those recovering from surgical procedures. These animals may already be physiologically stressed and, therefore, may require modifications in commonly used restraint methods and techniques. The stress of restraint may potentiate the preexisting condition.

In research facilities, the Institutional Animal Care and Use Committee should review proposed methods of nonhuman pri-

mate restraint in the experimental protocols and in the development of animal facility standard operating procedures. Input from all members of the committee is required, especially if the committee membership is such that it reflects diverse backgrounds from various research disciplines.

That restraint methods can affect many physiologic systems including the endocrine, nervous, cardiovascular, and other systems is well documented (Bouyer *et al.*, 1978; Butovskaia *et al.*, 1985, 1986a, b; Goncharov *et al.*, 1984; Hoffman *et al.*, 1968, 1972; Horstman and Banderet, 1977; Mason, 1972; Mason and Mougey, 1972; Mason *et al.*, 1973; Vrijmoed-deVries and Cools, 1985). The improper use of restraint methods can lead to chronic alterations in adrenocorticotropin (ACTH) release, growth hormone (GH) suppression, weight loss, alterations in the social order of the colony, or behavioral aberrations in the individual animal (Goosen *et al.*, 1984; Grynpas *et al.*, 1986; Kalin *et al.*, 1983a, b; Myers *et al.*, 1988; Perlov *et al.*, 1979; Pyke *et al.*, 1968; Walker *et al.*, 1990).

When more physically or immobilizing restraint methods or devices are employed, conditioning of the animals to these procedures to minimize stress and to allow humane care is required. This is accomplished by the training and adaptation of the nonhuman primate to the method selected (Barrow *et al.*, 1966). Ample time should be allowed to accommodate careful planning and implementation of this objective. The use of training and operant conditioning offers the advantage of allowing more flexibility in the selection of even less restrictive restraint systems. For example, training methods to minimize the need for restrictive restraints have been used successfully in the rhesus, baboon, and squirrel monkey (Morton *et al.*, 1987). Baboons were trained over a 2- to 5-month period to allow direct blood pressure measurement and to carry out self-administration of the drugs via the oral route (Turkkan *et al.*, 1989). Although the use of operant conditioning and training methods to facilitate less restrictive restraint appears to have the disadvantage of requiring added personnel and time in months to execute, the advantages offered include improved safety, enhanced well-being, humane care, and reduced stress on the animal.

Professional judgment and experience play important roles in the decision-making process for selecting each restraint method or technique. Adequate training of animal care staff, investigators, and veterinarians in the use of restraint methods is critical to the overall success of this process. However, the importance of each of these special concerns will vary with the actual purpose and expected duration of restraint. In an emergency situation, the safety of personnel and the animal become the highest priorities in procedures of any duration.

II. PHYSICAL RESTRAINT

Physical restraint methods are economical, safe, and effective if they are performed quickly and efficiently by experienced, knowledgeable personnel. Even though these techniques often involve the forceful manipulation of conscious animals, adverse effects on metabolic, endocrine, or physiologic parameters can be minimized or eliminated through the use of a training and preconditioning program for the nonhuman primate. These techniques may be used for the removal of a nonhuman primate from its cage for routine husbandry and care procedures or for experimental or clinical procedures such as physiologic recording, tuberculin testing, maintaining vascular access ports, or weighing (Altman, 1970; Hall and Flockhart, 1977; Lukas *et al.*, 1982; Moody *et al.*, 1970; Whitney *et al.*, 1967).

Because the use of physical restraint methods mostly involve conscious, unsedated animals, the risk potential for personal exposure of personnel to zoonotic diseases, bites, and scratches, and animal trauma and problems related to stress can be very high. These types of restraints often result in a high proportion of accidents in an animal facility (H. J. Klein, unpublished observation, 1993). For example, the improper handling of macaques using excessive force to pin their arms behind their backs during conscious restraint may result in fractures of the humerus (H. J. Klein, unpublished observation, 1994). Problems such as this can be alleviated through proper training of the animals and personnel involved. Clearly, because of the risks involved with the use of physical restraint methods, safety and prevention of injuries to personnel and the animal are crucial issues. Priority is always given to protection of the laboratory personnel when any physical restraint method is employed. Overall consideration should be given to the impact of the procedure on the well-being and health of the animal as well as the safety of the personnel involved (Klein, 1989).

The use of personal protective equipment (PPE) is essential when handling and restraining nonhuman primates. PPE commonly worn by those individuals restraining nonhuman primates include a properly fitting face mask, disposable latex or vinyl gloves, safety glasses with side shields, gown or laboratory coat, and long-sleeved shirts (Fig. C.1). The use of stainless-steel or Kevlar meshed gloves worn inside heavy leather gloves have been extremely effective in preventing deep punctures and lacerations inflicted by rhesus and African green monkeys during conscious handling for dosing and chairing procedures. (Figs. C.2 and C.3). Use of a long-sleeved shirt helps reduce the potential for scratches and minimizes the possibility of direct contact between the skin of personnel and nonhuman primate. The wearing of nonslip, steel-toed shoes is of particular value since these shoes help prevent foot injuries associated with handling large animals and heavy caging when the floors of nonhuman primate room are slippery and wet. Face shields should be worn when performing procedures with high aerosol potential. Large mobile Plexiglas or Lexan shields can be used to aid in protecting personnel. The shields are placed between the animal cage and research personnel to allow a safer approach. These latter two devices are helpful when working with experimentally infected animals to avoid splashes of water, urine, feces, or saliva from larger nonhuman primates and chimpanzees. Zoonotic and

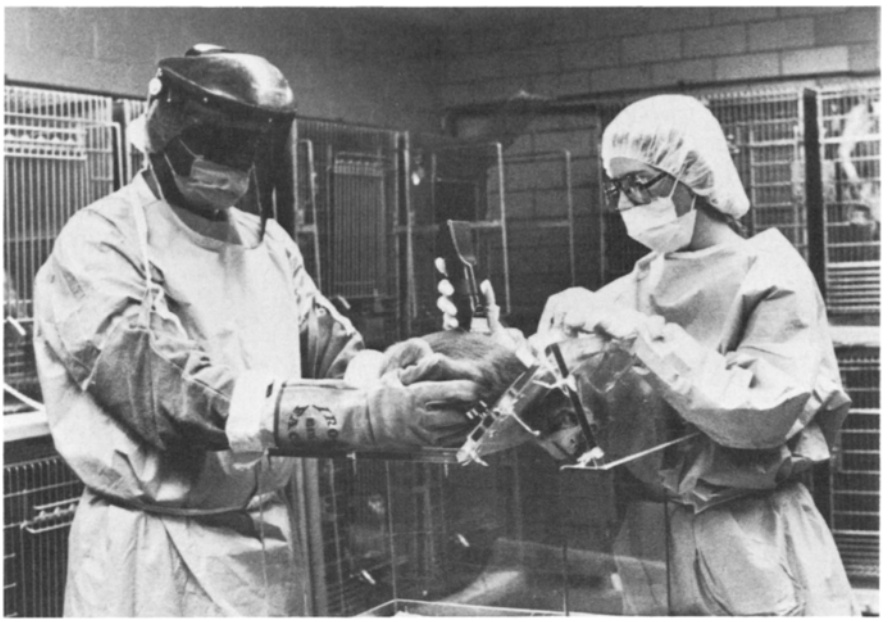

Fig. C.1. Proper personal protective equipment used when handling nonhuman primates.

physical trauma risks are reduced. A written institutional policy should be developed for the proper use of PPE when handling and restraining nonhuman primates. Such a policy serves as a uniform basis to administer safety, occupational medicine, and colony health programs of which the restraint and handling of nonhuman primates are integral parts.

III. RESTRAINT DEVICES

A variety of physical restraint devices exist for handling and restraining nonhuman primates (Anderson *et al.*, 1982; Bennet *et al.*, 1980, Blum *et al.*, 1983; Bourne, 1972; Buchholz and Montgomery, 1988; Byrd, 1979; Dost *et al.*, 1972; Fielder and Casmer, 1966; Findley *et al.*, 1971; Glassman *et al.*, 1969;

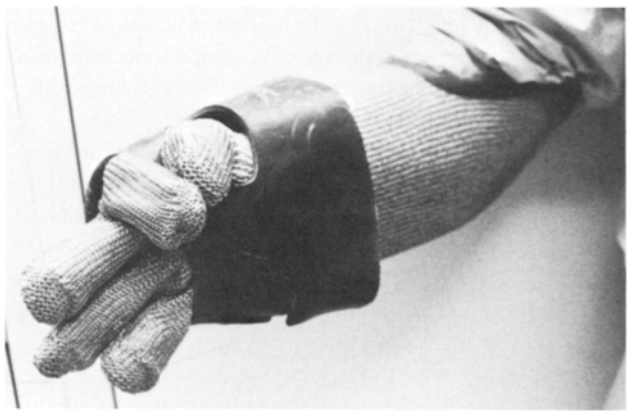

Fig. C.2. Stainless-steel mesh gloves with a leather shield for large nonhuman primates.

Fig. C.3. Kevlar mesh gloves with leather gloves for smaller nonhuman primates.

Hearn, 1977; Trost *et al.*, 1969). Some of the more commonly used types of equipment are described here. In general, desirable design and construction characteristics of these devices include safety features, durability, ease of sanitation, and simplicity and comfort for the animal and the laboratory animal care personnel. Typical materials used in manufacturing these devices include high impact plastics such as Plexiglas, Lexan, nylon, Delrin, polypropylene, and polyvinyl chloride. Stainless steel and aluminum are also utilized. Restraint devices should be limited whenever possible to use in individual animal rooms or with animals in similar experimental groups.

A. Cages

Cages constructed of stainless steel or aluminum with sliding squeeze restraint backs are the most commonly used restraint device for nonhuman primates (Fig. C.4). Squeeze-back cages allow for a safe, rapid, and relatively stress-free method of restraint for procedures such as routine examination and treatment, dosing, injection, and other basic animal care procedures. Squeeze-back restraint cages can be utilized for a variety of sizes of animals ranging from small New World species (e.g., marmosets, tamarins) to great apes such as the chimpanzee (Baker and Morris, 1980; Fielder and Casmer, 1966; Guilloud and McClure, 1972). In larger species, such as baboons and chimpanzees, geared mechanical, electrical, and hydraulic devices (Fig. C.5) provide an additional mechanical advantage in overcoming the strength of these larger species. Caution should be exercised when any squeeze mechanism is used to avoid injuries to the animal or operator as a result of trapping a body part of the caretaker or animal in the squeeze mechanism.

B. Nets

Nets often can be used in the animal facility for the capture of group-housed animals or escaped nonhuman primates. Nets have been used with particular effectiveness in catching smaller nonhuman primates held in group-housed breeding operations. Nets allow the captor to maintain a safe distance from the animal during capture to minimize the chance of bites or scratches. Nets can safely be used for various Old World species up to 3–3.5 kg (Fig. C.6) and for essentially all of the New World species. Lightweight nets should be used for the capture of smaller animals to prevent their injury (Fig. C.7). The stressful effects of excess struggling can be reduced if the nonhuman primate is allowed to calm down prior to its removal from the net. The calm animal can then be sedated or anesthetized with injectable drugs while in the net. Should the animal require handling without sedation, adequate personal protective equipment should be worn by an experienced person familiar with handling nonhuman primates.

Fig. C.4 A stainless-steel cage with a squeeze-back apparatus.

Fig. C.5 A squeeze-back cage with an electrically operated screw mechanism.

Fig. C.6 A capture net used for larger species.

Fig. C.7 A capture net used for smaller species.

Fig. C.8 A removable transfer chute.

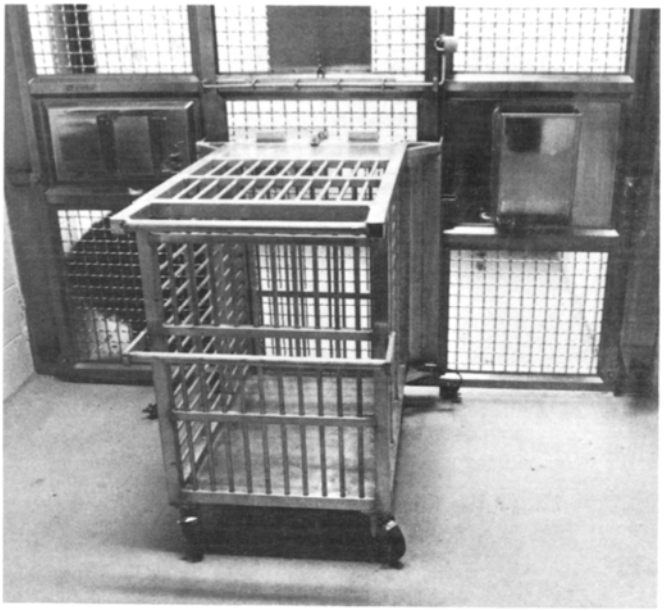

Fig. C.9 A mobile transfer cage.

C. Chutes and Transfer Boxes

Chutes or tunnels constructed of various materials can be used to interconnect nonhuman primate cages (Fig. C.8) Use of the chute facilitates a relatively easy transfer of various sizes and species of nonhuman primates either between cages or into another primary enclosure. Chutes can be constructed of heavy wire, stainless steel, or solid panels with sliding doors at each end and serve as a method of removing or segregating specific animals from an enclosure housing multiple animals (Bennett *et al.*, 1980; Fielder and Casmer, 1966; Guilloud and McClure, 1972; Swan, 1970). Many species adapt well to the use of this device and can be easily trained to use the transfer chute using food treats as a positive reinforcement.

Transfer cages or boxes (Fig. C.9) can be constructed to directly attach to cages to facilitate the transfer of animals to remote locations. Wheels can be added to these devices to facilitate the movement of larger nonhuman primates. Chutes and transfer boxes must have a heavy locking device that secures it to the cage or primary enclosure to preclude the chute or transfer box becoming disengaged during the procedure, allowing the animal to escape.

D. Stocks and Restraint Tubes

These devices are often constructed of transparent impact-resistant plastic materials such as Plexiglas. They routinely are used for the conscious dosing, bleeding, taking of samples, or weighing of nonhuman primates. The stock has been particularly effective for restraint during the flushing and maintenance of various chronic vascular access devices and implants in rhesus monkeys (Fig. C.10).

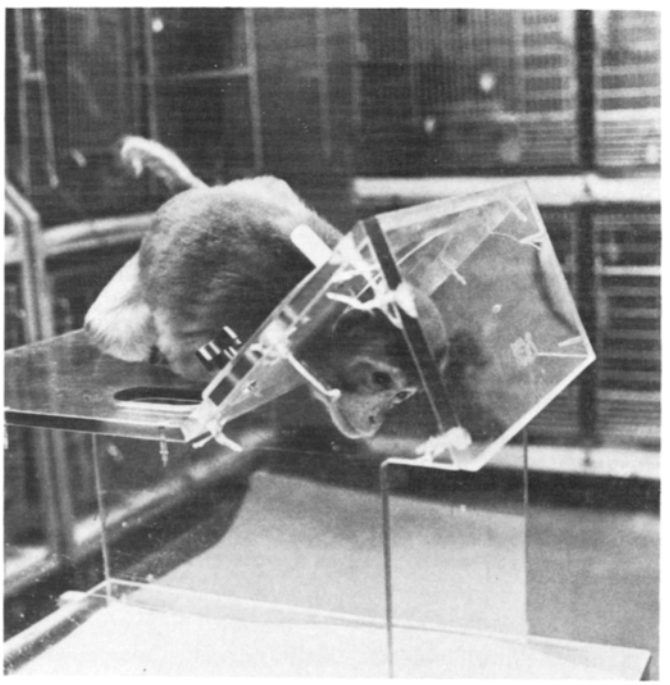

Fig. C.10 A plastic restraint stock for rhesus monkeys.

The stocks are designed to be comfortable for the animal and safe for the individual performing the restraint. Personnel safety is enhanced with the stock device shown in Fig. C.10 by the use of a shield placed across the front of the stock.

Animals can be easily trained to use these devices; however, stressful situations and distractions should be avoided to prevent animal escapes or injury to personnel using these restraint devices when these devices are in use.

Restraint tubes allow easy weighing and bleeding of juvenile rhesus or African green monkeys. Care must be exercised when new or stressful situations are encountered that might lead to injury of either the animal or personnel. These devices should only be utilized for short-term restraint since they severely restrict any animal movement.

E. Pole and Collars

The pole and collar method can be adapted for use in a variety of species, including the macaques, baboon, and squirrel monkeys (Anderson *et al.*, 1982; Salzen, 1989). With this device, each animal is fitted with a sturdy plastic collar to which a spring-clasp pole can be attached through the cage. The animal can be moved to various locations in the animal facility or placed in a specially adapted restraint chair which has a grooved yoke to accept the collar around the animals neck. The pole and collar method is particularly advantageous since the animals can be conditioned and trained to this system in a relatively short period of time. Routinely, macaques or baboons can be trained in as few as four or five 10-min training segments which combine the use of a so-called force training and positive reinforcement of behavior with fruit or food treats (Anderson *et al.*, 1982). Another advantage is that once an animal is trained, this technique is relatively stress free and still allows the safe handling and restraint of conscious nonhuman primate of larger sizes.

F. Restraint Chairs

Restraint chairs may be used in the research laboratory for a variety of purposes. Restraint chairs have typically been used to conduct ocular, cardiovascular, metabolic, neurologic, physiologic, pharmacologic, reproductive, and nuclear imaging studies using nonhuman primates (Casey *et al.*, 1975; Carlson, 1972; Caudhill, 1975; Fielder and Casmer, 1966; Hall and Flockhart, 1986; Henry and Bowman, 1971; Kuehl and Dukelow, 1974; Osborne, 1973; Rahlman *et al.*, 1976; Wolfe, 1974). Use of the restraint chair in these type of studies allows convenient and safe access to the animal while it is comfortably restrained in the chair (Fig. C.11). Chairing has been successfully utilized for a variety of New World and Old World species, including squirrel monkeys, marmosets, macaques, African green monkeys, and baboons (Blum *et al.*, 1983; Carlson, 1972; Dost *et al.*, 1972; Findley *et al.*, 1971; Golub and Anderson, 1986; Hearn, 1977; Lennox and Taylor, 1983; Milhaud *et al.*, 1980; O'Byrne and Morris, 1988). Special consideration should be given to animals with special conditions such as pregnancy (Fleischman and Chez, 1974; Golub and Anderson, 1986), infancy (Caudhill, 1977) or those with special anatomical or physiological needs (Howard *et al.*, 1971).

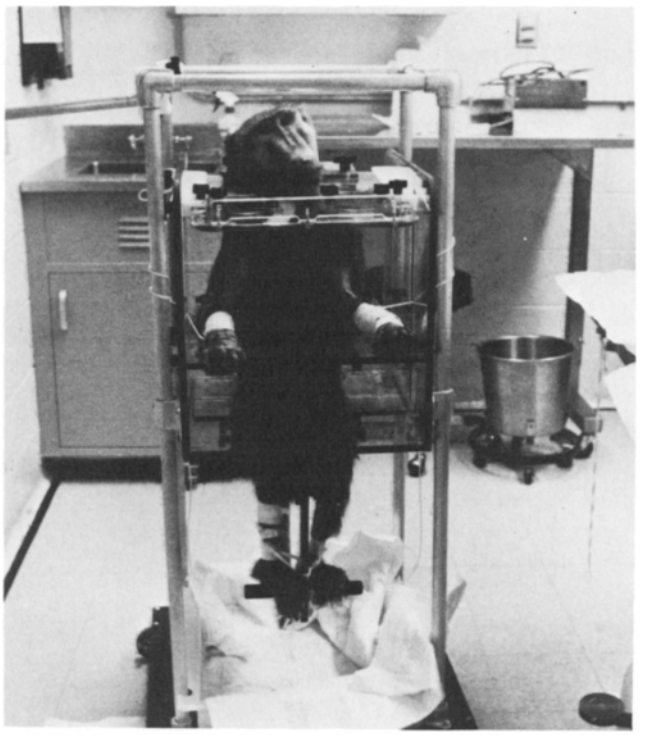

Fig. C.11 A nonhuman primate restraint chair.

Typically, restraint chairs are constructed of heavy-duty clear plastic material such as plexiglass, which is supported by a sturdy metal frame (Milhaud *et al.*, 1980; Sledjeski, 1969). The chairs should be designed to be sturdy enough to not only restrain the animal, but also withstand the rigors of routine washing and sanitizing procedures.

The restraint chair design should accommodate anatomical differences for the various nonhuman primate species so the animals are always comfortable (Braun *et al.*, 1968). This is especially true for the smaller New World species, but is also important for the macaques (Casey *et al.*, 1975; Nakamura *et al.*, 1982). Poor design that causes discomfort and stress to the animal can lead to invalid research data or, even worse, injuries to the animals. Injuries such as fractures or decubial ulcers may result. Adjustable restraint chairs accommodate for the anatomic differences between species such as position and length of the tail, position of the ischial callosities, crown–rump length variation, limb lengths, and postural variations (Mountjoy and Baker, 1974; Vidal *et al.*, 1986).

When chairs are used for restraint, the justification and duration of restraint should be closely scrutinized by the Institu-

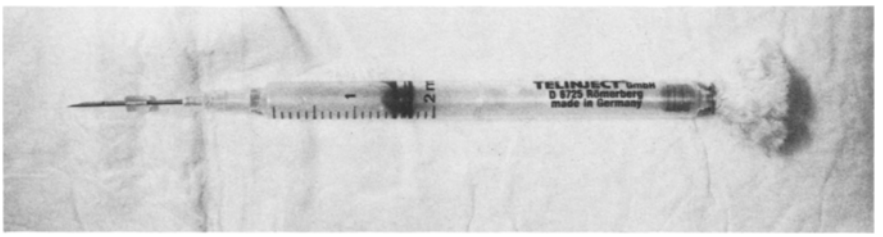

Fig. C.12. A blowpipe projectile syringe.

tional Animal Care and Use Committee. Currently, the U.S. Department of Agriculture regulations clearly state that chair-restrained nonhuman primates must be chaired for no longer than 12 hr before they are released for a suitable period of exercise.

It should be noted that many of the species previously mentioned can be easily adapted and trained to chair restraint over short periods of time. Animals can be initially placed in the chair for short intervals under close observation and then the restraint time can be increased incrementally. The use of positive reinforcement rewards will facilitate the adaptation. Water should always be provided during the chair restraint and feeding fruits and food treats should be performed using tongs or forceps to avoid injury to individuals caring for the chair-restrained animal.

G. Tether and Vest

Many species of nonhuman primates can be adapted to use the tether and vest system. Tether and vest systems have been used successfully in cynomolgus, rhesus, African green monkeys, and other species. This system, as described by various authors (Bryant, 1980; Byrd, 1979; Ducsay *et al.*, 1988; Lukas *et al.*, 1982; McNamee *et al.*, 1984; Mattson *et al.*, 1976), allows for the continuous administration of experimental compounds or withdrawal of samples via catheters enclosed in the tether protected by a flexible stainless-steel casing. These tethers can also contain lead wires attached to recording devices on the cage exterior allowing for continuous or intermittent sampling or data recording. The tether system is held in place by a nylon mesh or leather vest. The catheters are surgically implanted into easily accessible blood vessels such as the jugular vein, carotid artery, iliac vein, or iliac artery.

The tether and vest system offers many advantages such as the ability to continuously or intermittently dose, gather data, or obtain samples from either New World or Old World monkeys without seriously impeding the behavior or mobility patterns of the animal. The tether and vest system is very safe since direct handling and restraint of conscious animals are minimized; however, additional time, surgical support, manpower, and resources are required to prepare and monitor the tether system and animals over the course of the experiment.

IV. CHEMICAL IMMOBILIZATION FOR RESTRAINT

Circumstances in the animal facility may dictate that drugs be used to handle or restrain nonhuman primates. Drugs are used for restraint and handling procedures because of safety requirements, time constraints, and expedience or because of the experimental procedure or design. A variety of drugs may be selected, and they may be administered by various methods as described in the following discussions. Individuals should be familiar with the pharmacologic properties of the drug selected for use and be thoroughly aware of its limitations (Altman, 1970; Clifford, 1971; Wolfe, 1967).

When these agents are used, the nonhuman primate must be supported and closely monitored to avoid complications. In addition to providing adequate monitoring and support, many sedatives or anesthetics cross the placenta and some may be secreted in the milk and cause complications for the fetus (Whitney *et al.*, 1973). Complications also may occur on a delayed basis. The use of these restraint agents may have an indirect impact due to delayed effects on the animal or have an impact on the fetus or neonate (Guilloud and McClure, 1972). Gastric distension or bloat (Cohen and Bree, 1978) has been strongly associated with the prior use of anesthetics.

A. Drug Delivery Methods

A variety of drug delivery methods can be used to administer a chemical agent for immobilization and restraint. Intramuscular injections can be given very quickly with a handheld syringe if the animal can be physically restrained or confined in a squeeze-back cage. A pole syringe may also be useful if the animal is confined to a small area such as a cage, chute, or transfer box.

Capture pistols and rifles that project syringes and darts (Fig. C.12) are commercially available (Smith, 1981). Their range varies and they can be powered by compressed carbon dioxide, percussion caps, and compressed air. A blowpipe can also be used to deliver chemical agents (Dinnes, 1982; Melton, 1980). Major advantages of blowpipes include ease of use, minimal maintenance, silent projection, and less impact trauma.

A disadvantage of the blowpipe is its relatively short range (15–30 yards).

Some chemical agents may be administered orally. Ketamine hydrochloride can be effective when administered orally in dosages two to three times the intramuscular dose (Cohen and Bree, 1978; Vercruysse and Mortelmans, 1978). Restraint drugs for oral administration may be mixed in juice or fruit or may be squirted directly into the mouth of the animal. If restraint drugs are administered by the oral route, the effective dose and time of onset are far less compared with the parenteral route. Generally, the oral administration of drugs is not recommended unless other alternatives are not available.

B. Injectable Agents

A variety of restraint agents are available for parenteral use as immobilizing agents. The most commonly used drug for the chemical restraint of nonhuman primates is ketamine hydrochloride. Ketamine, a dissociative anesthetic, induces immobilization with some muscle rigidity. Swallowing, coughing, pedal, and corneal reflexes are maintained. An intramuscular injection at 10 mg/kg body weight will result in adequate restraint. Immobilization is usually produced within 5–10 min (Cohen and Bree, 1978). Dosages may vary with the species and overall condition of the animal.

Once immobilized, an indwelling intravenous catheter may be placed and the nonhuman primate can be maintained for longer periods through the intravenous administration of ketamine to effect 2–3 mg/kg body weight.

Ketamine may be administered in combination with other drugs to increase muscle relaxation and to enhance analgesia. Combinations include ketamine–xylazine, ketamine–acepromazine, and ketamine–diazepam. Ketamine–xylazine mixed in a 3 to 1 ratio (100 mg/ml ketamine, 20 mg/ml xylazine) administered intramuscularly at 0.1–0.2 ml/kg body weight provides immobilization with good muscular relaxation (Cohen and Bree, 1978).

Tiletamine hydrochloride, a cyclohexanone dissociative agent combined with zolazepam hydrochloride, a nonphenothiazine pyrazolodiazepinone tranquilizer, can be used for chemical immobilization. Administered intramuscularly at 2–10 mg/kg body weight, onset will occur in 5–12 min. It is useful for short-term immobilization; however, it is a Schedule III controlled substance (Cohen and Bree, 1978).

Etorphine (M99) is a Schedule II controlled drug related chemically and pharmacologically to morphine and is used in zoo and exotic animal practices. Administered intramuscularly to the nonhuman primate, it produces an acute onset, dose-dependent recumbency that can be reversed with diprenorphine (M50-50), a specific antagonist, at a dose of 30 μg/kg body weight. Diprenorphine is also a Schedule II controlled drug. Etorphine is extremely dangerous and may result in respiratory arrest if accidentally injected or splashed into the eye of a human.

Short-acting narcotics such as Alfentanil and Carfentanil may be used for chemical immobilization (Port *et al.*, 1984). The initial intramuscular dose is followed by an intravenous infusion; however, the dosages must be selected as appropriate for the species. Nalorphine, naloxone, and other morphine antagonists can be used for the rapid reversal of narcotic effects (Niemegeers and Janssen, 1983; Rosenberg, 1991).

Alphaxalone–alphadolone acetate (Saffan) is a steroidal anesthetic. Intramuscular administration at 10 mg/kg body weight can be followed with a continuous intravenous infusion of 40% Saffan mixed in an isotonic saline given to effect (Bengt, 1988; Box and Ellis, 1973; Cookson and Mill, 1983; Phillips and Grist, 1975).

If chemical restraint is required for longer than several minutes, consideration should be given to the use of parasympatholytic agents to decrease respiratory and salivary secretions. Commonly used cholinergic-blocking agents include atropine sulfate (0.02 mg/kg intramuscularly) and glycopyrrolate (0.005 mg/kg intramuscularly) (Cohen and Bree, 1978).

C. Restraint Monitoring and Support

During chemical immobilization, especially if of long duration, care must be taken to maintain the body temperature, hydration, and airway patency of the animal. Water-circulating heating pads, heat lamps, hot water bottles, and drapes or blankets may aid in maintaining body temperature. If the animal is young or debilitated, an infant incubator can provide supplemental warmth, and oxygen administration may be useful during the recovery phase. Electric heating pads should not be used and extreme care must be taken to prevent skin burns. The hydration status should be monitored during and after prolonged restraint.

With prolonged immobilization, endotracheal intubation is appropriate. Not only does this protect the animal from aspiration pneumonia in the case of vomition, it is also useful in an emergency or in treating respiratory depression. A resuscitation bag (Ambu bag) should be available in case assisted ventilation is required. With prolonged chemical restraint, the caloric needs of the nonhuman primate should be assessed. These needs can be met through total parenteral nutrition or administration of a high calorie gruel by nasogastric intubation (Hoffman *et al.*, 1968; Hobbs *et al.*, 1980). After feeding the animal should be observed closely for any signs of regurgitation/emesis and care should be taken to avoid aspiration.

Following recovery from chemical immobilization the behavior, appetite, hydration status, and urinary and fecal output of the animal should be observed daily. Appropriate nursing care should be provided on an as needed basis. It is extremely important to allow sedated or anesthetized animals to fully recover from the effects of the sedative or anesthetic before reintroducing them into a group housing situation. All animals should be carefully monitored to assure that all grouped animals have reestablished stable group dynamics and social interaction.

V. CONCLUSION

Various restraint methods are available for use with nonhuman primates. With an increased emphasis on reducing stress and assuring humane treatment, careful consideration should be given to the method selected. An increasing body of literature indicates that improperly performed restraint procedures can invalidate research data by disrupting the physiologic homeostasis of the animal. The development of innovative alternatives to the conventional restraint methods described in the literature should engender the development of improvements and alternatives which minimize some of the adverse effects of various restraint methods used today. The importance of training and adaptation of the nonhuman primate to the restraint method cannot be overemphasized. This not only reduces stress to the animal but promotes safety and quality data collection. Suitable alternatives to restrictive restraint techniques continue to be developed (Morton *et al.*, 1987). Telemetry (Ducsay *et al.*, 1983; Lange *et al.*, 1991) and positive reinforcement training which allow conscious unrestrained sample collection or testing (Turkkan *et al.*, 1989) will find increased use in the nonhuman primate research laboratory. Restraint methods for nonhuman primates should continue to evolve as a safe, comfortable, relatively stress-free, and reliable way to utilize the unique resource nonhuman primates represent to biomedical research.

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PART D. SURGICAL MANAGEMENT

Bobby G. Brown and R. Brent Swenson

I. INTRODUCTION

Fundamental considerations for surgery in nonhuman primates do not differ substantially from those for surgery in other species. However, some specific conditions that are appropriately managed by surgical means are commonly encountered in captive populations. Veterinarians who work with nonprimate species will find that some surgical techniques may be different due to anatomic or other differences that are unique to nonhuman primates. This chapter cannot address treatment of all aspects of surgery in nonhuman primates. Therefore, those conditions that are relatively common and those techniques that have qualities that are unique to primates will be emphasized. Procedures involving the thoracic cavity are not commonly encountered except for specific research purposes. Human texts and research articles should be consulted for those procedures.

Since anesthesia, analgesia, and critical care are discussed in Part C, these subjects will not be duplicated in this chapter, except for some general comments and when specific interventions are indicated for a given disease or procedure.

II. PRE- AND POSTOPERATIVE CONSIDERATIONS

A. Preoperative Management

In general, the preoperative evaluation and management of nonhuman primates is similar to that for other species. However, there are some differences. Preanesthetic sedation is not generally employed since most surgical cases are induced with intramuscularly administered cyclohexylamines, such as ketamine hydrochloride or tiletamine. Inhalant or other agents may then be used for anesthesia maintenance depending on the kind of surgery being performed and the condition of the animal. In practice, when augmented by appropriate analgesic agents, such as opioids and/or sedative muscle-relaxing agents (e.g., benzodiazepines), surgical anesthesia adequate for most procedures may be achieved with supplementary doses of ketamine.

A preoperative concern that frequently arises, especially in experimental surgery, is the use of prophylactic antibiotics. There are few practices in nonhuman primate medicine and surgery that are adopted more inappropriately than this one. Extensive literature is available on the appropriate use of prophylactic antibiotics in human surgery (Bergquist and Murphey, 1987; Segreti and Levin, 1989). Although there is still substantial disagreement over some specific applications, the accumulated evidence clearly shows that for most uncomplicated, elective procedures that do not involve implantation of a foreign material, antibiotic prophylaxis has no role in preoperative patient preparation. When a foreign substance (e.g., a chronic intravascular catheter, a vascular prosthesis, joint prosthesis) is used, it is important to provide intraoperative levels of an appropriate antibiotic.

Choosing an appropriate antibacterial agent requires an understanding of the pharmacology of the agent being considered and an "informed guess" about the organisms most likely to infect the prosthesis. An antibiotic with a short half-life, such as Nafcillin, may need to be repeated during a prolonged operation. Each institution should have some history based on previous microbiologic studies of the antibiotic sensitivity patterns that are prevalent locally.

Contaminated surgery, such as entry into the gastrointestinal or urogenital tracts, would warrant prophylaxis to include intraoperative levels of antibiotics to be continued for no more than 24–48 hr, depending on the level of contamination. Liberal irrigation of the contaminated areas helps to flush away the contamination and keep the bacterial population low. The use of antibiotics in operations involving an established infection should be considered therapy rather than prophylaxis, and the duration of therapy depends on the response of the infection (Bergquist and Murphey, 1987). Although antimicrobial prophylaxis is generally accepted to be appropriate for certain elective procedures where a prosthetic device is already in place (e.g., dental procedures in a patient with an existing vascular prosthesis), this practice is a matter of convention and is not governed by good scientific data (Segreti and Levin, 1989).

B. Postoperative Management

If a need for intensive care is anticipated, it may be advisable to have a special recovery cage available, postoperatively.

However, in most cases, this is not necessary and might even be inadvisable if the animal must be reanesthetized in order to be moved to another cage. Some concern about the integrity of the surgical wound may exist since primates have well-developed digital dexterity. Generally, there is little or no loss of wound integrity even though the animal may manipulate the sutures. Well-placed, interrupted, subcuticular sutures will provide sufficient holding strength to keep the skin edges apposed even with the loss of the skin sutures. The authors have noted that the nonhuman primates most likely to open their incisions are the macaques and great apes, especially *Macaca arctoides*, and *Pongo pygmaeus*. When wound manipulation or self-mutilation occurs, the use of phenothiazine or buterophenone tranquilizers, postoperatively, may be beneficial.

Self-mutilation may also be a manifestation of postoperative pain. Although it is difficult in many cases to properly assess the need for analgesics, selected drugs should be administered at proper intervals. For example, the use of meperidine twice daily is not appropriate practice since its analgesic effects last only for 3–4 hr and leave the animal with 16–18 hr of unrelieved pain. As with antibiotics, it is important to understand the pharmacology of the agent used and to match it with the nature of the pain being produced. Often, the combination of a nonsteroidal, anti-inflammatory drug and an opioid can achieve potent and effective analgesia for surgical pain.

III. ABDOMINAL PROCEDURES

A. Anatomical Characteristics

Most of the common major surgical conditions encountered in primates involve abdominal structures. With few exceptions, the relevant anatomy is not practically dissimilar to that encountered in companion animals. In most apes, extensive natural adhesions involving much of the large bowel and the body wall are considered normal anatomic findings. Such adhesions must be divided to permit thorough abdominal explorations. These adhesions may occasionally interfere with laparoscopic examination or surgery (Bush *et al.*, 1978).

A vermiform appendix is present in the great apes and the gibbon. Although uncommon, appendicitis has been reported (Ruch, 1959) and should be considered as a cause of acute abdomen syndrome. The uterus of primates is pyriform and is normally located in the pelvic cavity. It is not uncommon for some degree of adhesion to be present caudally between the ventral surface of the uterine body and the dorsal surface of the bladder. Even when full, the urinary bladder is flaccid when compared with the canine or feline bladder. The placenta of the rhesus monkey (*Macaca mulatta*) is usually bidiscoid, but may be single in as many as 22% of animals (Chez *et al.*, 1972).

In the great apes the close proximity of the last rib and the iliac crest makes a flank approach to the kidney technically difficult, particularly for the right kidney. In general, for renal operations, a midline approach in nonhuman primates is easier since the kidneys in monkeys are more free floating than in canines and are more accessible via this route. On the other hand, the spleen in great apes if firmly attached to the left body wall and diaphragm, and is most advantageously approached via a left subcostal incision.

B. Obstetrical and Gynecologic Procedures

1. Cesarean Section

Cesarean delivery of the fetus is one of the more common clinical operations performed in nonhuman primates. The procedure is indicated in cases of dystocia, incongruent fetal size and maternal pelvic diameter (Aksel and Abee, 1983), placenta previae, placental abruption, and pregnancies of dams known to injure or kill their infants. For an elective cesarean section, it is vital to time the operation when fetal maturity is sufficient to permit extrauterine survival. Estimates of gestational age are easier when a reliable conception date is available, but this is often not possible with nonhuman primates. In such cases, the gestational age must be estimated using radiographic or sonographic imaging (Newell-Morris *et al.*, 1980; O'Grady *et al.*, 1978). Fetal respiratory maturity may be accurately assessed by measuring the lecithin/sphingomyelin ratio in the amniotic fluid, obtained by amniocentesis. A ratio of 2 is indicative of fetal pulmonary maturity, a ratio of 1.5 indicates immaturity, and an intermediate ratio alerts the clinician to potential neonatal respiratory distress (Golde, 1988). The cesarean section may be performed using (1) the classical approach of a long vertical incision in the uterine fundus or (2) the low cervical approach in which a smaller, horizontal incision is made in the lower uterine segment after reflection of the urinary bladder from its attachment to the uterine fundus. The classical approach is technically easier and faster, and may be preferable for those individuals who do the procedure infrequently (Mosdol, 1976). In addition, the classical approach avoids the risk of accidental extension laterally of the low cervical incision into the large vessels of the uterus. The advantages of the low segment procedure include the minimization of drainage of potentially infected fluid into the abdomen; lessened blood loss from the thinner, less vascular lower segment wall; and prevention of omental and bowel adhesions to the uterus (Benson, 1971). A disadvantage is that the incision tends to retract back under the brim of the pelvis during suturing due to the overall contracture of the uterus. The wall of the larger nonhuman primate (macaques, baboons, and apes) uterus is much thicker than dogs and cats. In chimpanzees it can be 2–3 cm thick and edges need to be overseen with a simple continuous suture to stop the bleeding before the incision is closed with inverting sutures. Excessive blood loss may occur before surgery, as in cases of placenta previa or placental abruption (Calle and Ensley, 1985), or intra-operatively. When this occurs, a whole blood transfu-

sion may be indicated. The blood groups of many nonhuman primates have been described (Socha, 1980), and typing and cross-matching of donor and recipient blood are recommended. However, in experimental transfusions of incompatible blood, both in rhesus monkeys and in baboons, decreased erythrocyte survival times, but not transfusion reactions, were observed (Socha *et al.*, 1982).

2. Fetal Surgery

Fetal surgery is usually performed for experimental, not clinical, reasons, and techniques for it have been described (Michejda *et al.*, 1980; Harrison *et al.*, 1982). The major complications of fetal surgery relate to early parturition and fetal death. Successful fetal surgery requires additional experience and care. Stark *et al.* (1989) reported a series of 10 chronically instrumented maternal/fetal baboons in which the surgery was performed between Gestational Days 130 and 140 (normal gestation is 170 days). They achieved a mean fetal survival of 9.3 postoperative days (range of 0–29) with seven vaginal deliveries and five live infants. These authors concluded that a continuous postoperative morphine infusion was more effective at preventing early uterine contractions than other putative tocolytic agents, such as inhibitors of prostaglandin synthetase, β_2-adrenergic agonists, calcium channel blockers, and magnesium sulfate.

3. Ovariectomy

The most common indication for ovariectomy in nonhuman primates is to control endometriosis. Endometriosis is the presence of endometrial tissue in locations where it should not be present, and has been described in other nonhuman primates (Merill, 1968; Strozier *et al.*, 1972; King, 1973; MacKenzie and Casey, 1975; Folse and Stout, 1978; Fanton and Hubbard, 1983; Schiffer *et al.*, 1984). In severe cases, the abdominal cavity may be filled with so-called "chocolate cysts," cystic structures containing endometrial tissue and the dark residue of previous episodes of hemorrhage which occur at the same time as normal menses.

Location and identification of the ovaries can be difficult because of the severe anatomic distortions that may result from the adhesions and cysts. In some cases, the uterus becomes so enlarged that it causes constipation due to mechanical blockage and pain similar to inflamed hyperplastic prostates in dogs and complete ovariohysterectomy may be the best solution. Other conditions such as cystic ovaries and torsions of the ovaries have been observed by the author as a reason for ovariectomy. The technique itself is basically the same as for the dog except for the more difficult exposure due to the pelvic location of the organ in nonhuman primates.

4. Uterine Surgery

Unlike the situation for humans, where there is a large incidence of cervical neoplasia, it is rare in nonhuman primates. Consequently, hysterectomy is performed less frequently, but may occasionally be necessary in endometriosis or for otherwise uncontrollable hemorrhage. A subtotal hysterectomy, in which the cervix is left in place, is most often performed. Because of the anatomical association of the uterus and urinary bladder in nonhuman primates, care must be taken to avoid damage to the ureters as they enter the trigone vesicae.

Endometrial curettage is most often indicated in nonhuman primates for persistent postpartum bleeding associated with retained placental fragments. In the immediate postpartum period, the cervix is dilated and effaced, making access to the endometrial cavity easy. A sharp or blunt uterine curette may be used. A systematic approach should be followed to assure that a complete curettage is accomplished. Oxytocin is generally administered postoperatively to promote uterine contraction.

Biopsies and/or cultures of the endometrium are infrequently required for clinical purposes, but may be needed experimentally. In nongravid great apes, the cervix must first be dilated to allow insertion of a suction biopsy curette. In the macaque, a transcervical biopsy cannot be easily accomplished due to the normally sigmoid shape of the cervical canal. Olson and Sternfield (1987) have described a technique for percutaneous needle biopsy of the uterus that may be useful in these species. The addition of the ultrasound to guide the biopsy should greatly increase the ease and efficacy of this technique.

5. Laparoscopy

Laparoscopy may be employed as a minimally invasive procedure to accomplish many goals that might otherwise require open abdominal surgery, such as ovum collection; gross examination of the ovaries, fallopian tubes, and uterus; directed biopsies; and others (Dukelow *et al.*, 1971; Jewett and Dukelow, 1973; Graham, 1976; Bush *et al.*, 1978). After 38–67 laparoscopies in each of 16 female rhesus monkeys, Mahone and Dukelow (1978) were able to obtain eight conceptions and six live births, and concluded that multiple laparoscopies were not detrimental to fertility.

C. Gastrointestinal Surgery

1. Acute Gastric Dilation

Acute gastric dilatation is a condition familiar to most primate clinicians (Stein *et al.*, 1981; Soave, 1978). The etiology of acute gastric dilation has been the subject of much speculation, but is still poorly understood. Often the affected animal is found dead and there is no opportunity for intervention. However, when the animal is still alive, it is nearly always hypovolemic. The tremendous abdominal distension restricts the respiratory tidal volume and interferes with normal venous return by direct mechanical compression of the caudal vena cava and hepatic portal system. In addition, there may be effects of

various vasoactive amines which may be of endogenous origin and exogenous products of intragastric bacterial activity.

There have been no reports of controlled comparisons of different management strategies for acute gastric dilatation. Therefore, only anecdotal descriptions are available. Treatment is aimed at restoring the vascular volume and pH with appropriate fluid and electrolyte therapy, decompressing the stomach either via a large bore gastric tube or surgically, and providing appropriate cardiovascular and respiratory support. Ideally, gastric decompression is accomplished by use of a stomach tube which is removed after the stomach has been successfully emptied. However, gastric contents may plug even large-bore tubes so that surgical decompression is necessary. Care must be taken to avoid entering the tympanitic stomach during the initial incision through the midline fascia. The stomach can then be sutured to the incision to prevent leakage of gastric contents into the abdominal cavity when the stomach is opened. In our experience, even with optimal management, the mortality may be high in advanced cases, and recurrence is relatively rare in recovered animals.

2. Gastroenteric Obstruction

The great majority of obstructive bowel disease in nonhuman primates is related to the ingestion of foreign bodies. A gastric trichobezoar has been reported in a chimpanzee (Nolan *et al.*, 1988) and a baboon (Butler and Haines, 1987). Phytobezoars are commonly seen in outdoor-housed animals. Foreign bodies of other types, especially stones, are frequently seen in outdoor-housed macaques. Most of these pass without incident, although it is not uncommon for stones to accumulate in the cecum and be associated with signs of illness. Although less common, intestinal adenocarcinoma has been reported in rhesus monkeys (Hubbard *et al.*, 1984) and its occurrence may be more frequent than the literature suggests. In our experience, virtually all cases of intestinal adenocarcinoma have occurred in the region of the ileoceco–colic junction. Surgical management of these conditions is not substantially different in nonhuman primates than in other domestic species.

Another common, but visible, cause of obstruction of the gastrointestinal tract is rectal prolapse. This is frequently associated with diarrhea and with "stress" related to handling or social conditions. Spontaneous rectal prolapse may often resolve without intervention if the animal is left quietly in a cage. When this approach is used, it is important to observe the animal for at least 24 hr to assure that a normal stool is passed after the prolapse has been reduced. In our experience, enemas of lidocaine gel appear to be beneficial in nonhuman primates and infant macaque primates that are too small to allow digit insertion. This helps to ensure complete unfolding and filling of the colon and rectum and also gives some relief to the urge to strain. Anal "purse-string" sutures are sometimes advocated for prevention of recurrence, but the efficacy of this technique has not been compared in a controlled way to reduction without sutures.

When indicated, a prolapse may be amputated, with reanastomosis of the healthy rectal tissue. Transfixing the prolapse with 3-inch spinal needles before the amputation will prevent retraction of the proximal stump into the pelvic cavity before anastomosis can be accomplished. Sometimes the rectum and distal colon are involved to such an extent that anastomosis is not feasible after resection of the necrotic portion. In such instances, we have found that an end colostomy is a viable salvage procedure for a valuable animal. However, problems with scalding due to fecal soiling of the skin around the stoma, gradual contracture of the stoma, and occasional prolapse of the colon through the stoma limit the usefulness of this procedure to valuable animals. When rectal prolapse recurs frequently, colopexy may be warranted to prevent recurrence.

D. Splenectomy

Splenectomy in the nonhuman primate will most likely be required in support of specific research projects, such as malaria or immunologic studies. However, we have seen two cases of idiopathic thrombocytopenia in chimpanzees which were poorly responsive to prednisone in which splenectomy was curative.

The technique in monkeys is similar to that in dogs or cats and may be easily performed through a midline incision. In small monkeys, such as *Saimiri* and *Aotus*, the spleen is rarely attached to the abdominal wall and has a long enough pedicle that it can be delivered through a 2-cm incision with a spay hook. The vessels can be ligated with two to four sutures and the spleen cut free. In the chimpanzee the spleen is firmly attached to the lateral abdominal wall and to the root of the diaphragm, making access difficult from the midline. A more expeditious approach is through a left subcostal, transverse incision. Care must be taken to avoid injury to the pancreas during ligation of the splenic vessels. We have seen accessory splenic tissue in approximately 20% of chimpanzee splenectomies. This tissue should be removed as well.

E. Hepatobiliary Procedures

Clinical hepatic or bile duct surgical diseases appear to be uncommon in nonhuman primates. Cholesterol gallstones have been reported in *Aotus* (Anver *et al.*, 1972), *Macaca* (Geistfeld *et al.*, 1977), *Papio* (McSherry *et al.*, 1977), and *Pongo* (Ruch, 1959). These may be either asymptomatic or associated with clinical cholecystitis. Clinical disease is treated by cholecystectomy, using standard human techniques (Schwartz, 1989).

Hepatic lobe herniation through the diaphragm has been described in a rhesus monkey (Dalgard, 1969). Although associated clinical signs were minimal, the hernia was successfully reduced via a subcostal incision. We have seen one case of a hepatic lobe diaphragmatic hernia in a squirrel monkey that was not repaired and remained asymptomatic.

Parasitic involvement of the liver such as hydatid disease (Ilievski, 1969; Goldberg *et al.*, 1991) may be present in recently imported animals. These lesions must be aspirated under direct visualization and infused with a scolicidal agent such as formalin, 30% sodium chloride, 0.5% sodium hypochlorite, or silver nitrate before attempted removal of the cyst.

We have seen multifocal, calcified radiographic lesions in the livers of newly imported baboons that were apparently due to *Hepatocystis* infection, and required biopsy to rule out mycobacterial disease.

By far the most common open surgical procedures done on nonhuman primate livers are experimental wedge biopsies. A rapid technique for wedge liver biopsy in the chimpanzee using a stapling device has been described (Nolan and Conti, 1980), but the "finger-fracture" method and mattress sutures for hemostasis also work well. We have found that where large sections of nontraumatized liver are required for hepatitic testing and reference materials, practically all of the entire left lobe can be removed by preplacing several mattress sutures along the proposed incision line. After all the sutures are in place, they can be rapidly tied and the specimen cut free with the scalpel. The cut should be made from both the cranial and the caudal surface with the tip of the blade angled toward the midline to produce a V-shaped flap. The two edges of the flap of liver can be quickly overseen with a simple continuous suture of size 0 chromic catgut with a large blunt needle. The catgut does not seem to cut through the friable liver tissue as readily as other sutures. This will effectively seal the edges and stop nearly all of the bleeding (B. G. Brown, personal observation).

F. Urinary Tract Surgery

Surgical conditions of the urinary system of nonhuman primates are infrequently reported. Uroliths have been reported in several species of nonhuman primates (Ruch, 1959; Stephens *et al.*, 1979), but they appear to be uncommon. We have seen one case, in a chimpanzee, where a ureteral stone passed after several days.

A perineal cystocele has been described in a cynomolgus monkey (Martin, 1982). It was repaired by suturing the vagina to the pelvic floor and the tissues surrounding the pelvic organs to the fascia of the pelvic inlet, but it recurred at the next pregnancy.

IV. MISCELLANEOUS PROCEDURES

A. Air Sac Infection

Air sacculitis has been reported in the baboon (Lewis *et al.*, 1975; Gross, 1978), the owl monkey (Giles *et al.*, 1974), the chimpanzee (Strobert and Swenson, 1979), and the organgutan (Guilloud and McClure, 1969; Clifford *et al.*, 1977). In addition, we have seen air sacculitis in the pig-tail macaque and the pygmy chimpanzee.

Bacterial organisms are usually mixed, gram positive and gram negative, aerobes, although single organism infections may be present.

Treatment is aimed at clearing the infection, preventing aspiration and secondary pneumonia, and preventing recurrence. Simple aspiration of the exudate, even when combined with local instillation of antibiotics, is not effective. Systemic antibiotics are not helpful in the treatment of simple air sac infection and should be reserved for cases of secondary pulmonary infection. However, aspiration and irrigation of the sac with saline, followed by local instillation of antibiotics, may be effective when repeated several times over a 1- to 2-week period (Strobert and Swenson, 1979). Ablation of the air sac of a baboon was curative (Gross, 1978); however, in great apes, the extension of the air sacs into the axillary space makes total ablation difficult or impossible. Infections that cannot be cleared by serial irrigation or ablation of the sac must be treated by marsupializing the air sac to permit chronic drainage (Guilloud and McClure, 1969; Clifford *et al.*, 1977). Chronic marsupialization prevents large accumulations of exudate in the sac that may be aspirated, but it does not prevent the chronic aspiration of small amounts of infected material. Eventually the lungs of most chronically marsupialized animals become colonized with multiple gram-negative bacteria, including *Pseudomonas aeruginosa*, and the animals die of chronic pulmonary disease or immune complex glomerulonephritis. Prevention of pulmonary colonization might be accomplished by early separation and isolation of the air sac from the respiratory tract. This is best performed after endotracheal intubation via a midline incision over the larynx to expose the opening of the air sac bilaterally into the larynx. The mucosa is circumscribed at each opening, and the inner and outer rings that are created are closed separately.

B. Inguinal and Umbilical Hernia

Taylor *et al.* (1989) reported the bilateral repair of inguinal hernias in an 8-week-old chimpanzee. The repair was accomplished, although there were no clinical problems associated with the hernia preoperatively. Inguinal hernia and umbilical hernia are common in primates, but are rarely associated with clinical problems. Asymptomatic hernias generally do not require repair. However, they do tend to become larger and a source of concern about possible bowel entrapment. When surgical repair is performed, the peritoneal ring should be dissected free of the surrounding inguinal tissue and the excess peritoneal tissue removed or reflected into the abdomen with a few simple, interrupted inverting sutures. The inguinal ring should then be reduced to the appropriate size with vest over pants type interrupted mattress sutures. The legs of the mattress should be placed at varying distances from the edge of the ring to avoid splitting the fascia (Abrahamson, 1989).

C. Musculoskeletal Problems

Orthopedic problems are common in nonhuman primate populations. Most of these problems are seen in outdoor-housed animals. Limb fractures occur as a result of animals leaping from climbing and perching structures or during capture activities. At greatest risk are young, growing animals. Many of the fractures that are seen are epiphyseal. The orthopedic management of fractures in primates is similar to that for small domestic animals, but relies heavily on internal fixation (Kehoe and Chan, 1986).

Wounds incurred in fights among socially housed animals occasionally involve the bones and result in osteomyelitis. Where antibiotic therapy is not successful, amputation of the affected part of the limb may be indicated. Contrary to the practice for dogs and cats, in our experience nonhuman primates usually benefit from leaving as long a stump as possible below the knee or elbow. In lesions involving the hand, if only one or two digits or part of the hand can be left, it can be used very ingeniously by the animal. The animals usually do well postoperatively, even when they are returned to social groups.

Osteosarcoma has been reported in primates, but most cases in rhesus monkeys at least involve the skull or jaw and may not be amenable to surgical treatment (Reed and Garman, 1977; Sembrat and Fritz, 1979). In one case involving the right humerus of a squirrel monkey, the animal remained in remission throughout a 20-month followup period after amputation (Reed and Garman, 1977). In another case involving the left humerus of a rhesus monkey, pulmonary metastatic lesions resulted in the death of the animal 18 months after the amputation (Sembrat and Fritz, 1979).

We have encountered a single case of aseptic necrosis of the femoral head in a chimpanzee that was treated by femoral head resection. The postoperative course was fair. After a 3-year followup period the animal has remained ambulatory, although with mild lameness.

D. Intravascular Catheterization

A very common experimental procedure that may have clinical applications involves the placement of chronic, intravascular catheters. Surgical techniques for catheter placement and management postoperatively have been published (Craig *et al.*, 1969; Byrd, 1979; McNamee *et al.*, 1984). Successful catheter placement and management require careful attention to surgical asepsis. Prophylactic antibiotics are appropriate. Catheters, in place, need to be regularly flushed and filled with heparinized saline (minimum of every 48 hr and preferably more often), if not used for continuous infusion. Silastic catheters are less thrombogenic than other materials, but their soft flexibility may encourage obstruction when they are employed at a point of limb flexure. In addition to the obvious potential for infection, chronic catheters have been associated with chylothorax (Olson and Anver, 1979), immune complex glomerulonephritis (Leary *et al.*, 1981), and amyloidosis (Doepel *et al.*, 1984).

E. Oral Surgery

Adult male nonhuman primates of virtually all species have long, sharp, dangerous canine teeth that can inflict devastating wounds on cage mates or human handlers. Consequently, some mechanism for disarming these animals is indicated. Extraction of the canine teeth has been performed, but this can result in marked distortion of the occlusion of the remaining teeth. Tomson *et al.* (1979) reported a root canal technique for blunting canine teeth without extracting them, and Henderson *et al.* (1977) reported a pulpotomy technique for removal of the tooth crown. We have attended one rhesus monkey with mandibular osteomyelitis that was not responsive to antibiotics, but was successfully treated by surgical resection of a portion of the left hemimandible, approached from the mucosal side. No prosthesis or graft was placed in the defect. The animal was able to eat solid food within a few days of surgery.

Schofield *et al.* (1991) described a procedure for disarming the canine teeth of adult male macaques and baboons that employed amputating the canine tooth below the gingival margin, recontouring the alveolar bone, and covering the vital root and bony defect with a broad-based mucosal flap graft from the adjacent buccal mucosa. The primary advantage of the procedure was to avoid complications such as periapical abscess formation after crown amputation with endodontic pulpectomy and sealing of the pulp cavity. A total of 66 teeth were disarmed in 7 baboons and 16 macaques using subgingival amputation. Potential pitfalls with the technique were discussed, but complications were not tabulated and no controlled comparison with endodontic techniques was made so the superiority of one or the other technique cannot be confirmed.

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PART E. SPECIAL TECHNIQUES

James Levin

I. INTRODUCTION

Specialized techniques of primate medical management are an eclectic collection of experimental or clinical procedures that are unique to primatology or are not routinely employed in the practice of primate medicine. Using these techniques will enhance the effectiveness of veterinary care or research with primates, while at the same time improving the quality of the research environment and the interpreted results. Specialized techniques have arisen from the need to provide diagnostic or therapeutic care to patients who are generally noncompliant. Medical care cannot be provided to patients who by their very nature resist our interventions. As a consequence, specialized techniques have been developed which provide access to primates and compensate for the feral behavior of these animals in the research environment.

Human and veterinary medical procedures may be amenable to use with primates because of the anatomical and physiological similarities with other species which makes them such viable research models. It must be remembered that primates are not domesticated animals and are not naturally inclined to cooperate. This means that they are a potential biohazard and safety risk to humans, and can do harm to equipment or themselves simply by exhibiting normal behavioral responses. The use of chemical or physical restraints, to prevent this scenario can alter physiology and confound ongoing research. Fortunately, primates can be trained to willingly accept many procedures.

The use of specialized techniques with primates can minimize confounding effects of stress or inappropriate behavior on the interpretation of research, while maximizing the quality and quantity of these results. Such specialized techniques therefore allow for access to physiology or models of disease which often cannot otherwise be studied. For example, central venous access can be provided as part of routine veterinary or human medicine to most patients. However, the noncompliant behavior of a primate makes this routine procedure difficult at best to perform in a conscious animal, and potentially an unsafe practice for the patient. In a primate, central venous access can only be safely achieved through the use of a technique that is designed to prevent the primate from tampering with the system and unintentionally injuring itself.

The application of specialized techniques to primate clinical care or research is limited only by the creativity and imagination of a clinician. The driving force for the development of these techniques is the desire to apply existing medical or research practices to an uncooperative primate without confounding the results by the complexities of the procedure. The procedures which have been developed are for administration of clinical or experimental therapies, tissue and fluid sampling, physiologic monitoring, and specialized imaging techniques.

It is desirable in any situation to administer therapies to animals in a manner that is minimally stressful. Historically, clinicians or researchers have attempted to administer therapeutics with cunning or force. Examples of such techniques are hiding medications inside fruit or on jellied breads and manual restraint devices for oral or intravenous administrations.

II. ADMINISTRATION PROCEDURES

A. Venous Access

Emergency or intensive care often requires rapid venous access to initiate life-saving therapies in response to changes in primate homeostasis. Providing venous access for drug or fluid administration can be problematic during a physiologic crisis. Rapid intravenous catheterization is vital, but difficult, during an acute hypotensive crisis or shock. This is especially true in neonatal primates. Standard techniques include percutaneous peripheral or central venous catheterization procedures, or peripheral vein cutdowns (Kanter *et al.*, 1986). Indirect intravenous infusion via the intraosseous method into the marrow cavity is a technique that works well when standard methods prove unreliable. In small or medium-sized primates, the technique involves aseptic insertion of an 18-gauge hypodermic needle into the tibia approximately 1–3 cm lateral and distal to the tibial tuberosity (Berg, 1984). The technique has been successfully performed in adult or large primates using a 14-gauge hypodermic needle inserted into the medial malleoli. Emergency intravenous access should be achieved in less than 5 min to be effective. The technique is effective for rapid intravenous access for administration of fluids and drugs until alternative intravenous access can be obtained.

Repeated venous access or access for moderate intervals can also be problematic. The difficulty of infusion therapy may be in locating a site for venous access and then maintaining the intravenous line. Monkeys are inquisitive by nature and will quickly remove a catheter line if they can reach it. Restraint imposes the problem of continued sedation if chemical methods are used, or the superimposition of stress on an ill patient if manual methods are used. An interesting alternative is the use of rigid protective materials over sites of indwelling catheter placement. Both thermoplastics (Rose *et al.*, 1983) and fiberglass casting materials (Conti *et al.*, 1979) have proven to be effective at maintaining catheter retention, eliminating the need for a rigid whole body restraint of the primate. When venous access is difficult, particularly when repeated procedures are necessary for daily fluid therapy, alternative sites should be considered for use. Larger primates, such as macaques and baboons, have bilateral superficial lateral coccygeal veins that are easily accessed and amenable to repeated cannulations (Stickrod and Pruett, 1979). The use of this venous site is as effective as standard sites of peripheral vein catheterization and delays the need to use standard cutdown procedures until no other sites are accessible.

B. Enteral Administration

Typically, the objective of a study will require the administration of a material of interest on a chronic basis. This may be accomplished by using enteral or parenteral routes of administration. Enteral administration can be accomplished through dietary formulation or surgical models of gastrointestinal catheterization. Diets have been specially formulated for a variety of purposes. Studies of diseases such as atherosclerosis, which have an important dietary component, could not be performed without the ability to prepare diets that are highly specialized and controlled. The long-term administration and evaluation of potential carcinogens could only be studied in primates fed specially formulated diets (Adamson, 1972).

An interesting application of dietary formulations is the use of gelatin as a vehicle to administer food, vitamin supplementation, or therapeutic drugs to marmosets (Pereira *et al.*, 1986). These animals were often malnourished when first placed in captivity due to inadequate dietary intake and differences between natural and formulated diets. Many dietary supplements were offered, but gelatin-based diets were the most successful. Gelatin turned out to be ideal as a dietary supplement because it is readily available, easy to prepare, high in protein, and can be mixed with varied constituents to make a fortified diet. The ability of marmosets to survive in good flesh for 3 years when fed this type of a diet suggests the utility and success of this approach.

A more difficult problem is obtaining long-term access to a patient for therapy or research when oral administration cannot be achieved. Enteral administration in this situation is best accomplished by the use of a chronic gastric or intestinal catheter which can be exteriorized and accessed through a tether device or protective jacket (Lukas *et al.*, 1982). Silastic catheters can be implanted surgically into the stomach or intestines with minimal difficulty and are well tolerated by the primates. Intragastric catheters can be maintained for use in studies that span more than 1 year.

C. Parenteral Administration

Parenteral administration can be a more difficult task to accomplish. Typically, exposure to a body fluid space, such as intravascular (arterial or venous) or extravascular (intraperitoneal, subcutaneous, or intrathecal) spaces, must be established to facilitate the administration of materials for an extended period of time.

1. Vascular Spaces

The intravenous administration of drugs for extended dosing regimens is greatly facilitated when central venous access is availed through a chronic catheter (Lukas *et al.*, 1982). The animal is subjected to only one minor procedure instead of repeated venipuncture or indwelling catheterizations, and these catheters can be maintained for very long studies (>45 months). This is especially true in neonatal animals cared for with intensive medical management. The intravenous administration of fluids and parenteral feedings are lifesaving and may require this form of access to be continued until the animals can be weaned. Arterial access can be important in studies of organ metabolism or delivery of cancer chemotherapeutics (Phillips *et al.*, 1982). The ability to deliver drugs or metabolites into an artery which feeds one organ or tissue provides for specific application of a drug to a target site to increase biological specificity and activity, while reducing nonspecific toxicity or deactivation by other organ systems.

2. Extravascular Spaces

Access to extravascular spaces can be a useful tool for research or medical management of primates. The ability to deliver a hormone in a controlled fashion into the subcutaneous space can be a valuable research tool. The subcutaneous administration of some hormones such as gonadotropin-releasing hormone are biologically effective (Schmutzler *et al.*, 1988) and avoid the inconveniences associated with chronic venous catheterization (Ruiz de Elvira and Abbott, 1986). Insulin can be administered chronically into the peritoneal cavity and retain biological activity. The delivery of drugs to other extravascular spaces may be important in overcoming physiological barriers. The delivery of drugs or metabolic precursors into the cerebrospinal fluid space can be very important in chemotherapeutics or drug and metabolism studies involving the central nervous

system (McCully *et al.*, 1990). Similarly, systems have been devised to place a catheter in the vitreous cavity of the eye (Miki *et al.*, 1985). This affords the unusual opportunity to administer ocular therapeutics repeatedly or continuously for up to 18 months (Ishibashi *et al.*, 1986).

Administration procedures into the body may be performed directly through an administration port connected to a catheter or might involve the use of a sustained release device. Such devices may use solid-phase materials which degrade slowly over time or osmotic and mechanical pumps. Solid-phase materials offer the advantage of a single administration procedure for long-term therapeutic management. These procedures are very well tolerated by primates and are associated with a very low incidence of inflammation or infection. However, few materials possess the long-term stability necessary to be amenable to this type of formulation.

Materials which maintain stability for shorter intervals must be administered with pumping mechanisms which have shorter life spans or are refillable. Osmotic pumps, such as the Alzet pump (Alzet Corporation, Palo Alto, CA), are very useful for delivery of materials for periods of up to 2–4 weeks. These pumps are relatively inexpensive, and are lightweight and reliable. They can be implanted subcutaneously or placed externally in a backpack. However, they do have a limited life span and must be replaced to allow for continued administration of therapeutics.

Mechanical pumps overcome this limitation. Pumps have long life spans and can be filled as often as necessary to maintain viability of the infusate. Implantable drug pumps, such as the Infusaid pump (Infusaid Corp, Sharon, MA) can be used to infuse material into any fluid space or cavity. They are implantable and they recharge themselves simply by the process of refilling the reservoir. These pumps are significantly larger than osmotic pumps and the advanced technology they provide is also more expensive. However, they offer extreme utility in primate research and therapeutics if the pump size and cost are not a limitation. Larger pumps are available from other manufacturers which can reside in vests or jackets, or can be connected to the animal via a tether and swivel apparatus.

III. COLLECTION PROCEDURES

A. Introduction

The collection of biological samples and data is an activity made increasingly difficult by the nondomesticated nature of primates. Much work has gone into the development of specialized techniques for the collection of biologic samples and data to overcome this limitation and to allow these animals to live in an environment which places the least constraints on their captive life-style.

The collection of biological samples is key to the evaluation of the medical or physiologic status of a primate. The method of sample collection can have a significant impact on the results obtained following sample analysis. For example, the use of ketamine hydrochloride as a form of chemical restraint was found to interfere with interpretation of carbohydrate tolerance testing (Streett and Jonas, 1982). Castro *et al.* (1981) considered ketamine suitable for endocrine, metabolic, and cardiovascular studies. Similarly, normative hematology or serum chemistry results are always interpreted in light of collection methodology.

B. Vascular Access

Vascular access has received the most attention in the development of specialized techniques for primate medical management. Many systems have been devised to collect blood samples that do not require vigorous physical effort or chemicals for restraint. The simplest systems allow for collection of blood samples with minimal restraint whereas more sophisticated methods allow for sample collection whenever required.

The simplest systems for blood collections are designed to obtain samples efficiently, while reducing stress and discomfort to the animals. Short-term blood sample collection can be accomplished with standard techniques such as multiple venipunctures or through special techniques using vascular catheterization. Venipuncture is usually performed with animals chemically sedated or physically restrained. Improvements of standard venipuncture techniques have centered on minimization of restraint. Initial work was based on the need to collect blood samples sequentially with minimal disruption to the normal routine of primate colonies. The training of monkeys to the use of adapted restraint devices has been used effectively for this purpose. Monkeys can be trained to enter devices or chambers that apply gentle restraint, and they will present limbs for venipuncture (Phillippi-Falkenstein and Clarke, 1992) or blood pressure measurement (Turkkan, 1990). Venipuncture was described as being accomplished without removing animals from the colony, without exciting dominant individuals, without expending an unreasonable effort by caretakers, and without noticeable effects on group dynamics (Bunyak *et al.*, 1982).

Other forms of physical restraint require that animals be removed from their normal housing. These include chair restraint and pole and collar techniques (Anderson and Houghton, 1983). Both of these are described in Part C of this chapter. However, each offers advantages and disadvantages compared to venipuncture of sedated monkeys, and should be considered as viable alternatives. Used alone, or in combination, these techniques have proven invaluable in research or diagnostics of pregnant rhesus monkeys (Golub and Anderson, 1986), physiologic monitoring of organ system function (Gavellas *et al.*, 1987), measurements of metabolism (Dempsey *et al.*, 1986), and in behavioral research (Schmidt *et al.*, 1989).

Short-term catheterization has been cited as being advantageous to multiple venipuncture. The advantages of short-term catheterization techniques are access to specific venous sites and avoidance of complications of multiple venipuncture. Com-

plications associated with repeated venipuncture include infective thrombosis, thrombophlebitis, and phlebothrombosis (Bree *et al.*, 1982). Additional advantages are elimination of pain and stress to the animal, ease of use, and increased efficiency of blood sample collection (Flynn and Guilloud, 1988). Medical grade catheters can be purchased or made from a variety of materials, including polyethylene, polyurethane, silastic, and Teflon. These techniques require that monkeys be restrained for the placement of percutaneous catheters and for their continued use. Restraint can be in the form of chemical sedation or physical manipulations. The site of catheter entry is prepared aseptically, and a catheter is introduced into a peripheral vein either through or over a trocar or needle. The catheter can then be advanced to a desired location in the vascular tree. The technique can be used in conjunction with imaging devices, such as a fluoroscope, to collect venous blood leaving individual organs such as the brain (Carmel *et al.*, 1979).

Chronic access to the vascular system or other fluid compartments is a more complicated affair. To obtain fluid samples, catheters must be surgically implanted into a vessel or compartment, and exit through the body for fluid collection. Potential catheterization sites include arteries, veins, lymphatics, cerebrospinal fluid channels, exocrine glands, and secretory organs. Chronic access systems consist of a catheter component for entry into blood or other fluid compartments, and a collection devise such as an injection port or tether apparatus.

Chronic catheters are usually made from the same medical grade materials used for indwelling or percutaneous catheterization devices. The most typical use involves venous access for chronic therapeutic administration (Hoeprich *et al.*, 1982) or long-term sequential blood sample collection (O'Byrne, 1988). Arterial access can also be achieved and is very useful for sequential cardiovascular evaluations (Ternes *et al.*, 1983). The techniques involve surgical exposure of a desired blood vessel, with implantation and advancement of the catheter until the tip comes to lie in a desired location. The catheter is then secured into the blood vessel, ensuring hemostasis and preventing unwanted intravascular catheter movement. Once the catheter is secured to fascial tissue for strain relief, it can be tunneled through tissue layers and skin at an exit site conducive to the intended use of the catheter. The technique has proven useful and adaptable to such diverse primate species as marmosets (O'Byrne, 1988), baboons (Coelho and Carey, 1990), and macaques (Lotz and O'Neill, 1979; Scalese *et al.*, 1990). The technique has also proven useful in studies involving pregnant rhesus monkeys and their infants (Bailey *et al.*, 1981).

Complications of chronic catheter implantations have mostly been related to exposure of catheters and infections. The catheter exit site through the skin creates the potential of an exposed catheter for prying fingers or teeth to destroy, and to infection (Burrows, 1982). Every system of chronic catheterization must protect the catheters and catheter exit site. Catheter trauma is disruptive to research and may require additional surgical procedures. More significantly, a broken catheter, especially an arterial catheter, can result in exsanguination and death of the primate. Fortunately, the chronic catheter systems devised are all designed to minimize these risks.

The more typical problems of chronically implanted catheters are related to loss of patency, thrombus formation, and infectious processes. It is not uncommon to have inflammation and exudate at the catheter exit site through the skin. Most of these are self-limiting infections that pose little risk to the patient. Occasionally, these infections may migrate along the catheter, and fistulous tracts will develop to provide exit for purulent material.

Studies with catheters in nonprimate species clearly indicate the need for meticulous sterile procedures when handling catheters and a significant reduction in incidence of infection when procedures to prevent catheter contamination are strictly followed (Burrows, 1982). The use of prophylactic antibiotics at the time of surgery can also be useful in reducing the incidence of infection by commensual organisms, but is not a substitute for rigorous adherence to aseptic catheter care.

Thrombus formation is also typically seen as a result of chronic vascular catheters. The most likely cause of thrombus formation is mechanical damage to endothelium by the rigid catheter. This would suggest that more pliable materials such as silastic may be superior for this application. It has also been suggested that thrombus formation may be a predisposing risk factor to bacterial infections and sepsis. Timely catheter flushing, attention to asepsis, and the use of heparinized flush solutions can greatly lengthen the life span of a catheter.

Bacteria can migrate to the tip of the catheter once introduced through the catheter lumen by poor compliance with aseptic procedures for catheter care. Typical bacterial species isolated from catheter infections include *Staphylococcus, Klebsiella, Serrati, Pseudomonas, Proteus,* and *Enterobacter* species as well as various fungi (DaRif and Rush, 1983). Once a catheter is colonized with bacteria, it must be removed to eliminate the bacterial infection. Antibiotics can be employed to control the infection, but rarely will they be able to eliminate the bacterial infection with the catheter remaining in the blood vessel. The result of bacterial infections can be localized thrombus formation, with formation of vegetative lesions and emboli (Scalese *et al.*, 1990), or generalized septicemia and other sequela (DaRif and Rush, 1983). Vegetative lesions on heart valves and endocardium, fatal hemorrhage, abscessation, and visceral infarcts have all been reported as a consequence of the catheter sepsis. Other less typical problems associated with chronic catheterization of blood vessels include immune complex glomerulonephritis (Leary *et al.*, 1981), chylothorax (Olson and Anver, 1979), and secondary amyloidosis (Doepel *et al.*, 1984).

C. Fluid Collection

Once access to a blood vessel or other fluid compartments is achieved, a device must be employed to allow for controlled fluid sample collection. Techniques for this purpose include backpack systems with injection caps or stopcocks, vascular ports, and tether apparatus.

1. Indwelling Catheters

The simplest system would employ a catheter with a reusable cap on the externalized terminus. Early designs employed catheters that were sealed and coiled in a subcutaneous pocket closed with nonabsorbable suture material (Bailey *et al.*, 1981). This system was usable, but required sedation or restraint to access the catheters, and was limited by infections of the subcutaneous pocket. Two adaptations improved on the design. First, the development of backpacks proved useful in the reduction of infections by eliminating the subcutaneous pocket and the need for sedation to access the catheters. Second was the adaptation of injection ports or stopcocks to the catheter terminus. This capped off the system and allowed the catheter to be used without exposing the system interior to the environment.

2. Vascular Access Ports

Vascular ports were developed for the chronic administration of cancer chemotherapeutic agents that were cytotoxic (Dalton, 1985). The severe tissue reactions that developed locally at the injection site were a limitation to the use of these therapeutics. The compromised immune status of these patients necessitated the need to have central venous access without violating the protection to infection provided by skin. The result was a device that can be implanted under the skin, connected to a catheter which can be placed in any vessel or body cavity. The port itself is a rigid elastomeric base with a siliconized rubber septum. The rigid base facilitates anchoring to subcutaneous tissues and makes identification of the rubberized septum by palpation easy. The device has proven useful in primates for the administration of liquids, blood, or other fluid sampling and as an interface to the cardiovascular system for physiologic measurements or monitoring.

3. Tether Systems

Tether systems were designed as an alternative to chair restraint or telemetry (Byrd, 1979). Chair restraint is not well tolerated by all monkeys, and no monkey can be left in a chair restraint without alterations in physiologic systems. Telemetry circumvents the problems of physical restraint, but does not allow for fluid administration or sampling. The primary advantage of the tether is the measurement of physiologic function, or administration and sampling of fluid compartments in conscious, minimally restrained monkeys (Carey and Cooper, 1983). It therefore eliminates the complications introduced by physical or chemical restraint, while providing a superior system to obtain samples, administer treatments, and monitor physiology.

Tether systems consist of three basic components. First, there is a backpack which protects the catheters as they exit the body and provides a site of attachment for a flexible cable. Next, a flexible metal cable protects the catheters as they travel between the monkey and the cage exterior. Finally, there is a swivel at the cage interface which allows the flexible cable to spin freely without twisting the catheters contained inside. A second swivel can be employed at the catheter terminus to extend the catheters to stationary devices. Both catheters and wires can be contained within the tether for fluid collection or administration, or direct and transduced measurements of electromagnetic functions. The system is usually contained on a single cage. A tether system has been devised which allows four occupants the opportunity to engage in species-typical social behavior (Coelho and Carey, 1990).

Tethers have been employed for a variety of applications. First used for direct measurement of cardiovascular function in baboons (Byrd, 1979), their application has been greatly expanded. They have been used as an interface to access arterial and venous systems for administration of therapeutics, blood sample collection, and cardiovascular evaluations. Other uses include collection or administration of fluids from the central nervous system (Morton *et al.*, 1987) and the secretory glands and organs (Sopelak *et al.*, 1983). Multiple catheters are often employed (Bryant, 1980), and double-lumen catheters have been shown to work effectively (Quabbe, 1982). The limitation for fluid collection or administration is the technical ability to place a catheter in a desired location and the number of catheters that will fit through the system. Pregnant monkeys have been attached to tether apparatus to monitor both maternal and fetal functions (Ducsay *et al.*, 1988). Electrical measurements have included electrocardiograms (Stark *et al.*, 1989) and body temperature measurements (McNamee *et al.*, 1984).

A well-designed tether program will include an acclimation phase prior to surgical instrumentation. This allows the monkey to adapt to wearing the backpack and adjust to the range of motion allowed by the tether and swivel. Monkeys are typically fitted into the jacket to gauge their acceptance to their situation. Animals which clearly exhibit intolerant behavior can be rejected for use at this time. Most animals adapt to the jackets and can then be attached to a sham tether. Sham tethering should continue for at least 7 days prior to surgical instrumentation. Most animals require only one period of acclimation and demonstrate their tolerance by resuming normal behavior and displaying no overt signs of stress (Morton *et al.*, 1987).

It has been suggested that although monkeys may display normal species-specific behavior after tethering, some animals may have persistent elevations in heart rate for greater than 1 month postattachment (Kaplan *et al.*, 1983). The ability of the β-antagonist propranolol to block this elevation in heart rate suggests that there may be persistent arousal of the sympathetic nervous system associated with the tethering of cynomolgus monkeys (Adams *et al.*, 1988).

Problems associated with the tether apparatus are related to physical system failures, jacket designs, and concerns related to acclimation of the monkey to the tether. The balance between creating a lightweight system which allows maximum mobility with the strength necessary to withstand physical abuse is criti-

cal. System failure can have a catastrophic impact on the tethered individual and on data integrity.

IV. PHYSIOLOGIC MONITORING

A. Introduction

Physiologic monitoring often demands that a wide array of parameters be measured in order to be able to evaluate complex biological systems. Indirect, noninvasive, or simple methods of physiologic monitoring are useful as screening techniques (Gerbig *et al.*, 1975; Kraft-Schreyer and Angelakos, 1985), whereas more complex systems require sophisticated measurements for meaningful analyses. Much effort has gone into the development of systems to measure primate physiology. Many of these measurements require the recording of direct or transduced electrical signals to understand function or control of an organ or system. Direct measurements of electrical functions of heart or other tissues can be made and amplified for analysis. Force or motion measurements can be transduced into electrical signals making possible a wide variety of system parameters available for evaluation. Irrespective of the parameter being monitored, the procedure must be performed either directly with a continuous wire or remotely with biotelemetry.

B. Direct Measurement Techniques

Improved technology has brought sophistication and miniaturization of devices for recording direct or transduced electrical signals. A large array of measurement devices designed for human application or medical research have clinical utility for the direct measurement of primate physiology and function. Electrocardiograms (ECG) have been monitored in sedated monkeys and have proven useful in the diagnostic and research armamentarium (Gonder *et al.*, 1979). Cortical-evoked potentials and brain stem-evoked responses have been measured and found useful in the evaluation of spinal cord (Cusick *et al.*, 1979) and cochlea function (Dobie and Kimm, 1980), respectively.

Transduced functions such as blood pressure and blood flow have also become accepted techniques in primate medicine (Astley *et al.*, 1979). Direct measures of electrical function of the brain (electroencephalogram), eye (electrooculogram), muscles (electromyelogram), and heart (electrocardiogram) can be performed on the conscious primate (Halpyrn, 1985; Pearce *et al.*, 1989). Transduced measurements include blood pressure and heart rate; respiratory rate; body temperature; fluid flow through blood vessels or other channels; and mechanical measures of force, pressure, acceleration, and location (Meindl and Ford, 1984). With the appropriate electrodes, ion-based measurements of pH, chloride, calcium, oxygen, and carbon dioxide are also possible.

C. Remote Measurement Techniques

The techniques used to provide remote measures are tether apparatus or telemetric devices. The primary benefits of using a tether system compared to telemetric measurement are the ability to provide catheters for easier repeated fluid administrations or sampling and the ability to calibrate direct recording devices on a timely basis. The direct electrical connection between the animal and the recording equipment also tends to provide a cleaner electrical signal. Telemetry offers the distinct advantage of being a fully implantable system. This provides for complete mobility of the primate, and will not limit expression of natural behavior. This has made telemetry useful for studies of behavioral activity (Line *et al.*, 1989). The freedom offered by telemetry has been suggested to produce less stress than the tether systems which are often used with small primates such as marmosets. This was evidenced by a lowering of the heart rate and a maintenance of normal blood pressure (Schnell and Wood, 1993). The elimination of wires or catheters having to pass through the skin eliminated the need for cleaning or handling of these devices and results in a reduction in the risk of infection and septicemia.

Telemetry does have its drawbacks (Rasmussen, 1991). Systems tend to be expensive relative to the cost of a tether system. The systems usually transmit information by radio waves, and there is a potential for signal interference and loss of data. The life span of an implantable device is limited by its power supply. Particularly in small animals, the small battery size required for implantation will reduce the usable life of the device. The size of the transmitter may also prevent its use in smaller animals or limit the number of channels available for recording different biological parameters. There are systems available designed as a backpack which allow battery changes and overcome size limitations for smaller animals. Backpack telemetry systems have also ben used with great success in larger primates such as baboons (Spelman *et al.*, 1991). The systems have been used effectively to perform integrated studies of physiology and behavior (Astley *et al.*, 1991).

V. IMAGING TECHNIQUES

Specialized imaging techniques that are utilized in primates have primarily been modified from human diagnostic medical procedures. These techniques allow for direct visualization of anatomy not usually observed during routine examination of primates, or the development of images that are representative of morphology or function of the subject.

Radiographic Techniques

Primates are not ideal radiographic subjects because of anatomical differences between species which can confound

radiographic interpretation. Additionally, it is often difficult to visualize abdominal structures in standard radiographic views due to a lack of intraabdominal fat which reduces organ resolution, and an often abundance of gastrointestinal contents which obscures organ visualization (Hoffmann and Silverman, 1975).

1. Pneumoperitonography

Pneumoperitonography is a technique which has been used safely in human medicine (Stevens and McCort, 1964) and veterinary medicine (Morgan *et al.*, 1975). When applied to primate medicine it is considered a useful diagnostic tool whether used alone or in combination with other contrast procedures.

The technique is relatively simple to accomplish safely. Monkeys are prepared for radiographic imaging similarly to the generation of survey films. Ideally, animals are fasted and sedated. The animal is secured, and the lower abdomen is shaved and surgically prepared. A soft plastic cannula is passed into the abdomen with the aid of a metal needle at point midway between the right inguinal region and the umbilicus. The catheter is advanced into the peritoneal cavity, and then aspirated to confirm placement. If blood, feces, or urine are collected or if a moderate amount of air cannot be easily injected, then the catheter is repositioned. Carbon dioxide is then administered into the peritoneal cavity at a slow rate until the abdominal wall is moderately distended.

The animal should be placed in left lateral recumbency so if carbon dioxide emboli develop, they will localize in the right atrium and be filtered by the lungs. Cardiovascular and pulmonary function should be monitored to ensure that this procedure has no serious effects on the patient. The monkey can be positioned to obtain lateral and anterioventral images of the abdomen. The carbon dioxide will be absorbed in 1–2 hr. Images obtained with this technique were considered to allow easier determination of the size, shape, and location of abdominal viscera. When used in combination with positive contrast studies of the gastrointestinal tract, the entire mucosal and serosal surfaces can be visualized. This technique is contraindicated in cases involving peritonitis, local abdominal infection, free abdominal fluid, gastric bloat and dilatation, or diaphragmatic herniation (Morgan *et al.*, 1975).

2. Planimetry

Total lung capacity is a basic measure of the functional status of the lung. Conventional techniques to perform this determination such as plethysmography or gas dilution measurements require specialized equipment and can take much time. In contrast, planimetric techniques are simple to perform and should correlate well with measurements obtained in humans (Pratt and Klugh, 1967).

This technique was used to determine the total lung capacity of baboons and was compared to results obtained from humans using a similar technique and plethysmography (McCullough *et al.*, 1979).

Baboons were anesthetized and intubated with an appropriately sized cuffed endotracheal tube. An image was obtained at maximal inflation of the lungs using manual inflation to 40 mm Hg. A planimeter was used to trace the outline of the radiographic lung shadows (Harris *et al.*, 1971). The authors of this report felt that there was an excellent correlation between total lung capacity and radiographic lung area over a 100-fold range of body weight and a 4-fold range of body height. This correlation was better for young baboons than aged baboons.

3. Pelvimetry

Pelvimetry of squirrel monkeys as a predictor of perinatal mortality is a good example of utilizing conventional radiography to provide information otherwise not attainable. Initial attempts at breeding squirrel monkeys were met with varying degrees of success (Wolf *et al.*, 1975). It was thought that the large size of neonates relative to the maternal body weight was resulting in an inappropriate number of nonviable pregnancies (Aksel and Abee, 1983). Bolivian adult squirrel monkeys were radiographed in a standardized manner 6 months postpartum to obtain lateral and anteroposterior images of the bony pelvis. The pelvic inlet, midpelvis, and pelvic outlet dimensions were measured and compared based on the outcome of parturition. It was concluded that the size of the pelvis was a determinant in the outcome of pregnancy. A 10% reduction in pelvic outlet size resulted in a 20% reduction in area of the birth canal. This may result in impairment of fetal descent through the birth canal and increase fetal head trauma during parturition. The actual size difference was 0.17 cm. This is too small a difference to detect by physical measurement. Applying pelvimetric analysis to a breeding colony would allow for the removal of animals who would have a high risk of nonviable pregnancy outcome, and allow for maximization of management efforts toward those animals which are most able to produce offspring.

4. Hysterosalpinography

Determining patency of fallopian tubes is a difficult task. Hysterosalpingography can be performed on primates even with the anatomical difference of a tortuous cervix (Parmley *et al.*, 1983). In many primates, the cervical canal is not straight, but has varying degrees of tortuosity depending on the species (Hafez and Jaszczak, 1972).

Monkeys were sedated, and the cervix was visualized with a nasal speculum. An 18-gauge, blunt tip needle was placed in the cervical canal and manipulated until it passed into the endometrial cavity. One milliliter of contrast material was injected into the uterus and a radiographic image was obtained. Good quality hysterosalpingrams were obtained and radioopaque material could be visualized in both fallopian tubes in 75% of the

monkeys. It was concluded that hysterosalpingography is a useful technique in the evaluation of tubal patency.

5. Nomograms

Neurologic studies with monkeys have demonstrated some particular anatomical differences from humans which potentially compromises the usefulness of some species. Cynomolgus monkeys have been shown to have a brain stem which moves down and forward during radiographic imaging when the head is flexed (Kennedy and Ross, 1980). This variability cannot be accounted for in the standard atlas of the brain of this species, nor can the brain stem be accurately fixed in a restraint device to ensure geometric relational accuracy. The development and use of a nomogram have overcome this problem. This nomogram uses contrast and plain radiographic studies to outline the brain stem and relate it to external immovable metal markers. The nomogram relates the brain stem and study target to bony landmarks which will be present on plain radiographs, which are identified and related to the metal markers. Location of the study site on plain films with the use of the markers allows for accurate measurements relative to the brain stem. Accuracy of generated coordinates can be easily monitored with plain films during the entire study. This technique allows for accurate and reproducible neurologic studies which overcome a potentially compromising anatomical difference between primates and humans.

6. Adjunctive Techniques

The use of adjunct techniques can improve the usefulness of radiographs obtained for clinical diagnoses or scientific research involving primates (Aitken, 1983). Angiography is a useful technique to improve on information gathered on conventional films. Accurate measurement of blood flow can be a useful diagnostic technique, as well as an important research tool (Wallenburg and Hutchinson, 1979). Many primate clinicians are comfortable with the use of contrast medium to visualize anatomic structure or function. The intravenous pilogram or excretory urography study is a good example of this use. Similarly, the use of contrast media in the cardiovascular system can provide useful information of blood flow and related anatomic structure and function. The experience of primate clinicians is that iodine-based contrast media is well tolerated in monkeys. The technique involves the placement of intravenous, intraarterial, or intracardiac catheters to allow visualization of a target structure after the rapid injection of a contrast media. Sequential radiographs are then obtained. Examination of the images allows for determination of normal or abnormal patterns of circulation. These circulatory patterns relate to structural anatomy and function which can be assessed based on the patterns of radioangiographic entry, dispersion, and disappearance of the contrast media.

The use of contrast agents in radiographic images produces a high contrast radiograph which details the outlined structure. However, one of the limitations of this technique is the occasional inability to discern the surrounding soft tissue structures due to their radiolucency. Often precise identification of structures cannot be determined and approximate locations of such structures are assigned. The use of implantable metallic clips, such as hemostatic clips, is useful in providing reproducible landmarks in primates for improved radiographic image analysis (Laitman and Crelin, 1980). The placement of implantable metal clips on internal structures provides an unmistakable reference point for the identification of soft tissue structures. The clips increase the visibility of soft tissue structures, particularly if these structures move during physiologic activity. Metallic clips are easy to handle and can be applied to organs such as the respiratory tree or gastrointestinal tract without requiring a surgical approach. The clips do not interfere with normal physiologic activity, and are most useful in the precise analysis of movement or moving structures. The clips can also be applied or implanted in bony structures for the identification of bony structures. The clips have been most useful in studies of analysis of bone growth and development.

7. Image Analysis

Image analysis of conventional radiographs allows for quantification of changes in sequential radiographs which may not be detected by the unaided eye. Subtraction radiography can be used to demonstrate changes in tissue density which might be obscured by overlaying tissues or anatomic structures (Ort *et al.*, 1977). The technique enhances slight changes in tissue density by subtracting images which interfere with viewing the structure of interest. The technique can be performed with computer-driven software to perform sophisticated analyses or with conventional films which are processed with a subtraction film mask (Lurie *et al.*, 1983). The technique has been shown to be useful in studying fine structures of cerebral vascular beds (Ort *et al.*, 1977) and changes in bony structure related to bone growth or disease (McWilliams, 1982).

8. Fluoroscopy

Fluoroscopy or similar devices which use lower intensity of roentgen rays to produce images are very useful in determining the location of catheters or other devices within a primate body (Blocker *et al.*, 1986). Whenever a diagnostic technique can be performed without incorporation of surgical methodology, then there is a benefit to both clinician and patient in terms of time, effort, and cost. Techniques which would permit sampling of intestinal contents include multiple surgical biopsies, stoma or fistula formation, creation of intestinal loops, or pharmacologic intervention with gastric lavage. These procedures are invasive, complicated, and prone to the development of complications. The use of a steerable catheter under fluoroscopic guidance

obviates the need for such difficult or invasive procedures (Ford *et al.*, 1989). This system can be used effectively to sample intestinal contents for bacterial or viral isolation, administration or sampling directly from the small intestines, collection of tissue biopsies, or measurement of immunoglobulin in intestinal secretions. The skill required to effectively utilize this technique is easily gained through practice with this device.

Fluoroscopy can be an invaluable aid in the detection of catheter and tube placement for the management of primate critical care. Resuscitation and maintenance of critically ill primates often involve the placement of endotracheal tubes, umbilical artery and venous catheters, chest tubes, nasogastric tubes, and Swan Gantz catheters. Tube and catheter placement must be exact and requires radiographic imaging for confirmation. Often a series of studies is required and a delay in image analysis can be life-threatening. The use of fluoroscopic or other roentgen ray imaging devices offers the ability to provide real time images that are easily analyzed. This can be a life-saving technique in the management of critically ill primates.

9. Other Imaging Techniques

More sophisticated imaging techniques have proven useful in primate medical management. Scintigraphy, computed tomography (CT) and nuclear magnetic resonance (NMR) imaging, and positron emission tomography (PET) scans have all been applied to primate diagnostics and research. Although these techniques provide information which may not otherwise be obtained, highly specialized equipment is required. The cost to obtain and operate this equipment is often a great disincentive to their application and may not overcome the justification for their uses.

a. SCINTIGRAPHY. Scintigraphy involves the use of a gamma-emitting isotope and scintigraphic image capture with a computerized gamma camera or detector. The technique is useful for hemodynamic analyses as well as for functional analyses of hard and soft tissues. A gamma-emitting isotope is selected based on radiopharmacodynamic effects most suited for the intended application. For example, labeled indium, xenon, and technicium are good gamma emitters with properties which enhance their usefulness for cardiovascular evaluations. Indium binds to transferrin when injected intravenously, restricting its distribution to the circulating blood pool. Indium has been shown useful in dynamic placental scintigraphy of pregnant macaques. It provides clinical measurements of changes in maternal blood flow which cannot otherwise be obtained without endangering the fetus (Skjöldebrand *et al.*, 1989). The technique was also used in primates to validate the human clinical experience. Xenon has been demonstrated to be a good indicator of blood flow and has been used to measure cerebral perfusion in conscious baboons (Sakai *et al.*, 1979) as well as cerebrum and skeletal muscle of sedated baboons (Marcus *et al.*, 1981). Technicium can be bound to cells or other materials, which then define its distribution pool. It can be bound to blood products for cardiovascular applications or substrates such as methylene diphosphate for studies of metabolic activity of bone.

Studies with vervets have demonstrated changes in the metabolic activity of bones after various treatments to enhance remodeling of bone grafts (Dormehl and Engelbrecht, 1984). The correlation of scintigraphic results with clinical and histologic results suggests the usefulness of scintigraphy for sophisticated and discriminate analysis of bone growth and response to injury in primates.

b. COMPUTED TOMOGRAPHY. Computed tomography imaging has been utilized with primates to study organ blood flow and to study strategies for improving this technique in human clinical medicine. The most common technique involves the use of xenon inhalation with CT imaging. CT imaging offers the advantages of being a noninvasive technique that is accurate in primates (Dhawan *et al.*, 1984), offers high spatial resolution, good anatomic correlation, and is easy to perform (Fatouros *et al.*, 1987). It also does not require the use of radiopharmaceuticals to generate an image. Xenon (Xe) is a freely diffusible tracer which readily crosses blood–tissue barriers (Gur *et al.*, 1984). It has a relatively high atomic number (54) and provides image enhancement when inhaled in low concentrations in oxygen. It is ideal to study time-dependent tissue concentration and tissue perfusion. The combination of Xe inhalation with CT imaging provides improved spatial resolution, and rapid serial imaging over time for quantification of blood flow rates in discrete locations. The technique has been used effectively in baboons and macaques to study blood flow in the cerebrum (Holden *et al.*, 1980), liver and kidneys (Gur *et al.*, 1984), lungs (Gur *et al.*, 1979), and midbrain (Drayer *et al.*, 1979, 1980).

c. NUCLEAR MAGNETIC RESONANCE. Nuclear magnetic resonance imaging techniques are less utilized in primates than CT imaging. Magnetic resonance imaging is effective in tissue characterization and in providing anatomical detail. In the fetus, it is considered to provide better soft tissue contrast than ultrasound (McCarthy *et al.*, 1985). To date, no known hazards related to its use with mammalian cells have been confirmed (Schwartz and Crooks, 1985). Initial studies using animals demonstrated the feasibility of this technique and its application to human medicine. Chimpanzees were early test subjects to demonstrate that blood flow measurement was possible and to correlate test results with the initial human studies (Battocletti *et al.*, 1979). The technique was later applied to the quantitation of total body water measurements and the estimation of fat contents in baboons (Lewis *et al.*, 1986). Pregnant rhesus monkeys were studied near term without ill effect to mother or fetus (de Podesta *et al.*, 1986). Mothers delivered normal fetus without signs of developmental anomaly or pathology as a consequence of the procedure. The images produced were considered to be improvements in diagnostics and to provide more information of clinical relevance.

d. POSITRON EMISSION TOMOGRAPHY. The positron technique has proven to be a useful adjunct to research. Studies in

monkeys have measured glucose uptake and metabolism in the brain (Brownell *et al.*, 1980), oxygen utilization by the central nervous system (Mintun *et al.*, 1984), cerebral blood flow (Larson *et al.*, 1987), cerebral and myocardial blood flow (Green *et al.*, 1988), and myocardial perfusion (Hack *et al.*, 1980). The noninvasive mapping of neuroreceptors is an exciting application. PET scans in the study of dopamine receptors in rhesus monkeys have demonstrated the utility of this application (Eckernäs *et al.*, 1987). The ability to perform *in vivo* autoradiography suggests that primates can be studied throughout their life without ill effect and can be monitored to detect changes in function from growth or aging. PET scans have been performed on pregnant monkeys demonstrating the safety to mother and fetus, and providing invaluable information on fetal–maternal interaction and metabolism (Lindberg *et al.*, 1985).

VI. FIBER-OPTIC TECHNOLOGY

Flexible fiber-optic technology has revolutionized the ability to image remote previously impenetrable locations. The morphologic similarities between humans and primates have allowed for the direct transfer and application of this technology. This has been especially true with the advent of pediatric or neonatal devices designed to be used in very small spaces.

The development of devices for imaging the respiratory and gastrointestinal tracts has proven useful in primate diagnostics. The flexible bronchoscope has proven effective in the imaging of airways, pulmonary function, and respiratory pathology (Muggenburg *et al.*, 1982; in the administration of therapeutics (Marsh, 1989); and in the collection of samples by biopsy (Strumpf *et al.*, 1979) or lavage (Cohen and Batra, 1980). The endoscope has gained much popularity as the instrument has become smaller in size and easier to manipulate. The endoscope has been used effectively to image and sample the upper and lower gastrointestinal tract (Blackstone, 1984). It has proven useful in marmosets and tamarins to study colonic disease pathology throughout life (Clapp *et al.*, 1987). The limitation to endoscope usage is the length and diameter of the instrument. Endoscope length limits the distance of penetration in larger monkeys. The diameter of the endoscope limits the size of tubular structures in which the scope can traverse. Similarly, flexible fiber-optic devices have been used to visualize urogenital systems. The endoscope, cystoscope, and nephroscope have been successfully used to image the urogenital anatomy of various larger monkey species (Bahnson *et al.*, 1988). The fetoscope was developed for fetal diagnostics and therapy prior to the introduction of ultrasound or other noninvasive modes of fetal imaging. Although the device can effectively image the fetus, it is associated with a greater risk of abortion than the other techniques (Mattison and King, 1983), and was mostly abandoned. However, the introduction of fetal laser therapy for treatment of *in utero* vascular anomalies (DeLia *et al.*, 1989) has renewed interest in this fiberoptic technology.

VII. ULTRASONOGRAPHY

Ultrasound imaging uses high frequency sound waves to create an image which is demonstrative of the reflective or echoic patterns beneath the detector. Ultrasound offers the advantages of relatively low cost per image, transportability of the equipment, and safety since ionizing radiation is not required to create an image.

The technology initially gained utility for the *in utero* detection and monitoring of pregnancy. The ability to estimate gestational age in fetal macaques (Nyland *et al.*, 1985; and Cho *et al.*, 1987), gorillas (Yeager *et al.*, 1981), and baboons (Farine *et al.*, 1988) has been a simple application for some time. It is reported that ultrasound gestational age estimates are accurate to within 2 to 4 days of actual gestational age, depending on the primate species involved (Farine *et al.*, 1988). The ultrasound scanning technique has even been adapted to chair-restrained animals who can be walked from their cage to the chair, scanned, and returned to their cage without the use of sedation. Fetal blood samples can also be obtained using ultrasound. Ultrasound-guided umbilical vein blood sampling is a minimally invasive technique which can provide a high quality blood sample without the necessity of fetal surgery (Lindgren and Lindberg, 1985). Ultrasound technology has also been used to replace invasive laparoscopic techniques to image the ovaries of rhesus monkeys to detect follicle development and ovulation (Morgan *et al.*, 1987). This application demonstrates that as operator skill and machine resolution continue to improve, the technology will no longer be considered special and will become part of the routine diagnostic imaging.

The cardiovascular application of ultrasound imaging has also proven useful in primate medical management. Echocardiography, or real-time ultrasound imaging, can be performed in two dimensions, M-mode, or B-mode. Two-dimensional imaging and M-mode analysis have been used to diagnose cardiac anomalies relating to structural or functional defects in a variety of primate species (Allen *et al.*, 1985). B-mode ultrasonography has been demonstrated useful in the diagnosis and assessment of atherosclerosis in nonhuman primates (Bond *et al.*, 1989). Continuous wave Doppler technology also uses sound waves to monitor cardiovascular function, but in a different manner than echocardiography. Doppler technology allows for the measurement of movement by quantification of reflected frequency shifts. When coupled with scanning ultrasound to measure geometry, and direct the ultrasound beam accurately, one can make accurate measurements of blood flow through specific vessels (Bosman *et al.*, 1988) or anatomical regions in primates (Antunes *et al.*, 1983). The sophistication of the scanners has greatly increased their utility, but the escalating cost has kept this technology unavailable to most primate clinicians.

The greatest potential of ultrasound may be an application which has received little attention in primate medicine: the use of ultrasound as an imaging tool to visualize visceral organs and structures in a dynamic state of activity. Ultrasound imaging is

considered a routine imaging technique in human medicine for abdominal pain and disease differentials; visceral abscess diagnosis; detection of gall stones and hepatobiliary diseases; and diagnosis of liver, pancreatic, prostatic, pulmonary, renal, testicular, thyroid, and urinary tract problems. Although all of these organs can be imaged in primates using ultrasound, there still must be much effort invested in establishing normal anatomical images as a reference source for the detection of clinical disease in primate medicine.

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PART F. CRITICAL CARE

Daniel P. Rosenberg

I. INTRODUCTION

There is no specific definition of critical care. In some instances entry into the emergency room can be the beginning and end of critical care. It may refer to the various intensive care units such as cardiac intensive care. It may also refer to therapy rendered in life-threatening situations, acute or chronic in origin. Critical care is terminology originating in human

medicine and has become a subspecialty (Shoemaker *et al.*, 1989). Veterinary medicine has begun to organize certain services under this cognomen in major teaching hospitals (Zaslow, 1984).

Critical care is action-oriented medicine. There is an immediate need for therapy. Rapid assessment of the condition of the patient is the first step in determining what treatment must be started. Information obtained from a quick, but thorough, physical examination is essential. The initial therapy will be based on these physical findings. During the physical examination blood and other fluids or tissue should be obtained to establish baseline laboratory results. The best possible care will depend on the ability of the clinician to institute immediate treatment that can be continuously monitored using both physical signs and sequential laboratory data.

A. Situations Leading to Critical Care

There are innumerable reasons and situations that lead to a primate requiring critical care. While preventive care should be the foundation of any primate facility's operation, there are always unforeseen conditions that may require critical care. The ability to provide critical care is a requisite to meet these problems at any time.

B. Natural Causes

The need for critical care stems from two general categories: natural and iatrogenic. In captive primates, the majority of critical care is devoted to the treatment of trauma and environmental problems, such as hypo/hyperthermia. The most frequent and worst trauma occurs in group-housed primates. However, even single-housed primates require critical care, frequently as the result of self-inflicted injury due to aberrant behavior.

Environmental causes, especially hypothermia, may afflict more than one patient and may require the involvement of many people at a facility. The need for critical care in these situations makes it imperative that many or all staff be familiar with emergency medical procedures, irrespective of their job descriptions.

Most primates requiring critical care fall into two categories. The first and most difficult is the "downer monkey" or a monkey that has been found prostrate, frequently by a caretaker or technician making routine early morning rounds. The majority of other monkeys requiring critical care are conscious and are brought in sporadically during normal working hours. The monkeys in the first group are severely depressed or may even appear to be dead and there is usually little or no history about the origins of their condition. They must be attended to immediately and will require constant attention throughout the first day. Those monkeys that filter in throughout the day usually have relatively straightforward problems and some information about their condition is available because they have already been seen periodically during routine daily operations of cleaning and feeding.

1. Hypoxia

Assessment of the depressed or unconscious primate can and should be done quickly, without chemical immobilization. Temperature, pulse, and respiration are basic data. If there is any sign of severe respiratory distress, this must be relieved immediately. A patent airway must be established. The oral cavity must be examined using surgical or other high quality gloves. Exposure to potentially lethal organisms in performing emergency medicine is high because one is invariably having direct contact with blood, saliva, etc.

Any blood, saliva, or vomitus must be removed immediately. Vomitus is by far the most dangerous because it not only blocks the airway, but frequently has or will be aspirated causing fatal pneumonia several days after the monkey may have been saved from asphyxiation. Material in the oral cavity can be removed using forceps and gauze pads. However, the most expedient and effective method for clearing the airways is to simply hold the monkey upside down by its ankles. This can be done easily by one person in all but the larger primates. Gravity can be far more effective than suctioning or endoscopy, both of which are rarely immediately at hand. If the monkey is very heavy it may require two or even three people to accomplish "inversion cleaning." If this is physically impossible due to the size of the primate, the patient may be securely strapped to an examination or surgical table and placed in an extreme head down tilt position. After inversion cleaning has begun, a more thorough cleaning of the nares and the oral cavity, including the cheek pouches, should be accomplished. If suction is available, it may be used to clear the upper respiratory tree. Physical stimulus to the primate may promote some degree of consciousness and there may be additional regurgitation. Always be ready to do another inversion cleaning.

As soon as the airway is cleared, the use of supplemental oxygen should be considered. There are several ways of delivering oxygen to a hypoxic primate. The simplest method is to run a tube from the oxygen source directly into the oral cavity. This allows easy access to the primate for monitoring mucous membrane color and capillary refill time. Another simple method is to surround the head of the patient with a clear plastic bag and run the tube into the bag creating a rudimentary, but effective, oxygen tent. With infant primates it may be necessary to place the bag around the entire body. Any oxygen tent must allow direct and rapid access to the patient. A more "high technology" method is to supply oxygen directly to the nares using a U tube similar to those used in humans (Anesthesia Associates, Inc., San Marcos, CA).

The most aggressive means of supplying oxygen is to intubate the monkey and use a ventilator. Although this is the most sophisticated method, it is cumbersome and requires equipment and skills that may not be readily available. The most practical use

of intubation is with a resuscitation or "ambu" bag (Airshields Inc., Hatboro, PA) that supplies room air and ventilation. An ambu bag can also be connected to an ordinary anesthetic face mask. Oxygen can be connected to the face mask instead of using a plastic bag. Practically speaking, face masks frequently are not effective when used on primates. They are designed for dogs and cats and usually do not fit well. Masks available from human hospitals suffer from the same drawback. However, an assortment of nose cones should be available because some times they may be effective and it is better to have as many options available as is possible.

2. Hypothermia

Concurrent with determining the airway status of a primate, one should immediately obtain the core body temperature. A fairly accurate status can be found by touch, color, and history. Most primates requiring critical care will be hypothermic. This is almost always true of outdoor "downer" monkeys, and nearly all moribund infants are hypothermic with the exception of those being housed in incubators. Hypothermia and hypoglycemia are the conditions to look for and remedy in most moribund primate infants.

A primate found down in its cage is frequently hypothermic. A preexisting condition causes the monkey to lose the ability to remain upright or perched, and the monkey rapidly loses body heat, even in a warm environment (Hey and Mount, 1967). The metal cage floor acts as a "heat sink" conducting body heat away from a recumbent primate (Elder, 1989).

While a patent airway is being established, the clinician must have a plan to treat hypothermia. Lack of a precise temperature should not stop preparations for immediate treatment. Using the standard normal temperature of 101.5°F as a benchmark, any moribund, cold to the touch primate can be assumed to have a core body temperature of approximately 90°F or less (Dietrich, 1980). The precise temperature can be measured many ways. A glass rectal thermometer can be inserted while a primate is being taken to a treatment room. The temperature of a moribund primate should be the first piece of data available to the clinician. Thermometers should be available in every treatment room, operating room, or other areas where primates are handled on a daily basis.

There are other thermometers that are very useful. One is the new inexpensive unbreakable plastic digital device (Basic Medical Products, Inc., Englewood, CO) that beeps as soon as it registers the body temperature. Another one measures the temperature of the tympanic membrane (Ototemp, Henry Schein, Port Washington, NY). It is now being adapted for veterinary use and may become very easy to use in primates. Once the initial temperature is obtained, it is imperative to monitor the effects of treatment. Probably the best instrument for continuous monitoring is an electric thermometer (Yellow Springs Corp., A. H. Thomas, Philadelphia, PA).

A sudden drop in core body temperature affects the metabolism of mammals in many ways. When treating hypothermia in primates the body's attempt to maintain an overall metabolic equilibrium must be kept in mind if therapy is to be successful. Core body temperatures below 80°F have been recorded and successfully treated by the author.

The goal of successful treatment is to raise the core body temperature slowly, but steadily until it approaches 100°F. It is imperative to remember that the number on the thermometer is only a number and reaching a predetermined "normal" temperature does not ensure clinical success. Overly aggressive efforts to raise core body temperatures to normal can be lethal. Response to therapy is the most important result and the thermometer should be used as a guide.

Some of the inexpensive, commonly used heat sources pose certain hazards and generally should be avoided. Electric heating pads, made for use at home, should not be used. Their thermal output is nearly impossible to regulate, and even if it is, the direct heat they apply to the skin can cause serious burns. These burns can be massive, but not recognized for several days after the pads have been used. Sloughing of full thickness skin may occur. The necrotic skin under an apparently normal, well-furred area may literally slip off the body.

In general the use of heat lamps is not recommended. They pose similar problems as electric heating pads because their output cannot be regulated. Heat lamps aimed directly at a cage raise the temperature of the entire living area and do not allow an animal to move to a cooler environment when the core body temperature reaches an acceptable level. Heat lamps are convenient and if they must be used, they should be placed a few feet away from the cage and should be aimed at only a portion of the living area and not turned directly on a recumbent animal. Small hair dryers do not effectively raise the core body temperature and the continuous blast of hot, dry air dries the mucous membranes.

The best heat sources are recirculating warm water pads (k-pads, Gaymar Inc., Buffalo, NY). If the core body temperature is 95°F or above, recirculating hot water pads should be sufficient to warm the monkey to a normal body temperature. A core body temperature of 95°F may be encountered during routine surgeries, and although below normal, it is not life-threatening unless there is a severe underlying condition, such as sepsis. The water pads and insulating towels should be used until the core body temperature reaches 100°F or above. If the monkey regains its normal temperature, but does not appear healthy, hypothermia is not the primary problem and the search for an underlying disease should be the focus of diagnosis and treatment.

If the core body temperature is 90°F or below, immediate, aggressive measures must be taken. There is a significant amount of literature describing theories and techniques for treating hypothermia (Mills, 1973). These methods are described in human emergency handbooks and also can be found in veterinary texts (Dietrich, 1980; Kirk and Bistner, 1990;

Meier, 1984). Most of them are esoteric, including warming blood by creating an arteriovenous fistula, cardiopulmonary bypass, peritoneal dialysis, and thoracotomy with mediastinal lavage. In humans it has been found that although these invasive methods increase the success of resuscitation, they do not offer overall improved survival rates (Ledingham and Mone, 1980). These methods are neither available nor suitable for treating primate hypothermia.

In clinical use the most practical and effective technique is warm water immersion. This can be very effective, but certain precautions must be taken. Do not use water that is hot to the touch. You should be able to put your hand and arm into water that does not require "getting used to" but is soothing. Measuring water temperature is of little value. If water temperature is above your comfort level, it can easily damage primate skin. Hypothermic primate skin is vasoconstricted and cannot accommodate high external temperatures that under normal circumstances could be dealt with through functional heat exchange mechanisms.

Warm water immersion is the quickest means of reaching normal body temperature. It is tempting to raise the core body temperature as fast as possible; however, it is essential that it be done gradually, which requires continuous monitoring. When using warm water immersion therapy, rewarming should occur no faster than 0.5°F per minute (Shoemaker *et al.*, 1989). For example, if the core body temperature is 90°F, it should take at least 30 min to reach 100°F, the very upper limit the core body temperature should reach. If rewarming is proceeding too rapidly, the primate should be removed from the water bath and wrapped in warm water pads until the process is brought under control. With careful monitoring, a slight decrease in the core body temperature may be observed after it has been raised to near normal temperature. This decrease, or "after drop," is the result of the cold blood returning from the decreased peripheral vasoconstriction (Shoemaker *et al.*, 1989). Do not panic and resume immediate reimmersion. The key to successful treatment of hypothermia is controlled, gradual increase in the core body temperature.

A rapid rise in body temperature can cause metabolic disaster in several ways (Shoemaker *et al.*, 1989). The error most often caused is temperature "overshoot." When the core body temperature reaches approximately 95°F, it almost always rises exponentially and within less than 2 min can easily reach 105°F or more. Although the monkey may begin to recover consciousness, its metabolism cannot handle the demands placed on the various organ systems. It must be understood that hypothermia is a relatively gradual process, one that the body is remarkably well equipped to handle. As body temperature goes down, blood pools in the most critical organs such as the heart, liver, and lungs. All efforts are directed to protecting these organs and most importantly the brain. As body temperature drops, so does the carbohydrate requirements of the brain. This protects the brain against hypoxia and other crucial insults (Shoemaker *et al.*, 1989). Essentially the brain and other internal organs go into a protective hibernation. When the core body temperature suddenly rises the brain instantly demands all of its physiologic nourishment, especially oxygen and glucose. Irreparable brain damage may occur. Also, core organs become flooded with cold, acidotic blood from peripheral limbs due to the vasodilation that occurs with extremity rewarming. All of these processes combine to put impossible demands on vital organs. The temperature overshoot that occurs is a measurable manifestation of the metabolic disequilibrium that is a result of rapid rewarming (Civetta *et al.*, 1992; Shoemaker *et al.*, 1989). A moribund hypothermic primate that appears to be recovering may suddenly die when its core body temperature overshoots normal or even approaches normal too fast.

Before or during immersion therapy, it is essential to place an indwelling venous catheter for warm fluid therapy. This may be very difficult before vasodilation begins. Do not delay immersion therapy, but begin insertion attempts as soon as a vein appears.

Fluid therapy serves two purposes in hypothermia therapy. First, warm fluids help restore circulation and assist in increasing temperatures everywhere, especially deep vital organs (Civetta *et al.*, 1992; Shoemaker *et al.*, 1989). The fluids should be warmed before infusion. The fluids should be about the same temperature as the immersion water.

The second important purpose of fluid therapy in hypothermia is to restore intravascular volume depletion (Shoemaker *et al.*, 1989). During the drop in basal metabolism that occurs in hypothermia, many intricate processes, including decreased renal tubular resorption of free water, increased cold-induced capacitance of blood vessel volume, and decreased secretion of antidiuretic hormone, lead to intravascular volume depletion. A condition known as "cold diuresis" occurs when core body temperature falls (Elder, 1989; Farmer, 1992). This leads to acute tubular necrosis in fatal cases of hypothermia (McKean *et al.*, 1970). The infusion of fluids is necessary to refill the blood vessels. Like rewarming, this must be done with restraint. Any isotonic fluid solution can be used. An accurate tabulation of the amount of fluid infused must be kept to avoid fluid overload. When the heart loses its ability to pump at maximum capacity, fluids may pool in the lungs. Once the heart is pumping near normal, pooled fluids may be pushed into the brain causing cerebral edema sometimes referred to as "cold edema" (Stone *et al.*, 1956). The restoration of fluid volume must be guided by clinical signs and knowledge of fluid behavior in the hypothermic patient. It is difficult to rely on the usual standards of fluid therapy that apply to treatment of dehydration.

In addition to the mainstays of warm water immersion and recirculating hot water pads, there are some other useful tools. One is the hot water bottle. Several of them, each wrapped in a towel to prevent damage to the skin, can be placed around and under the patient. A "high technology" version of the hot water bottle are gelatin packs that are made to be heated in microwave ovens (Safe and Warm, Inc., Boulder City, NV). They are effective, but due care must be exercised when using them because

they can become very hot. Major drawbacks to their convenience is that they tend to explode when used repeatedly and their temperature is not easily monitored when they are being heated. Another "high technology" source of heat are pouches that generate their own heat when "popped" (Prism Technologies, Inc., Chicago, IL). These are quite safe, inexpensive, and long lasting (several hours) and are available at camping stores. They should be kept in storage with the hot water bottles. Also, synthetic, lightweight blankets that are very thermal efficient can be bought at camping stores (Moore Medical, New Britain, CT).

Along with high and low technology procedures, do not overlook the benefits that can be derived from vigorous rubbing of the torso and extremities. Use your hands and dry towels. Sometimes this stimulation alone can revive moribund hypothermic primates, especially infants. "Dead" infants have been revived with a good rubdown.

Small space heaters may be useful for warming a cool treatment area and also can be useful in maintaining appropriate ambient temperatures for revived primates that are returned to single cage housing. Better yet is the use of disposable warm water heating pads placed in the cage bottom (Baxter Pharmaseal, Valencia, CA). Heating pads are better than space heaters because they cannot harm the patient. A monkey in a cage with a pad can get off the pad if it becomes too warm, whereas they cannot escape direct blasts of hot air from a heater. The "disposable" pads, plastic or paper, can be cleaned and reused many times to hold down costs. Also, there are hookups that can connect one pump to two or three pads simultaneously (American Hamilton, Cincinnati, OH). This may be a lifesaver when multiple cases of hypothermia must be treated.

In summary, hypothermia is frequently the ultimate cause of death of many primates, large and small. One should have the basic knowledge and tools to treat it effectively. Be aggressive but be extremely vigilant. This is one disease where the cure can be worse than the disease.

3. Hypoglycemia

So far the initial assessment of a primate requiring critical care has focused on two fairly obvious physical condition: hypoxia and hypothermia. There is a third condition that frequently must be dealt with: hypoglycemia. Detection of the first two requires only the physical senses of the clinician. Hypoglycemia must be definitively diagnosed using laboratory techniques. This requires obtaining blood, which can be very difficult even for the most proficient phlebotomist. However, there is a simple diagnostic approach to detect hypoglycemia, assuming that it is present. Hypoglycemia frequently occurs with hypothermia (Civetta *et al.*, 1992). Although the two are not directly related to each other, by the time a monkey is found down it usually has low blood glucose. When comatose humans are presented to the emergency room, frequently little or no useful history is available, and like monkeys, they cannot speak for themselves. Therefore part of the general treatment plan used in an emergency room for coma is to administer boluses of two medications and use response to therapy as a diagnostic tool. A bolus of 2.0 mg naloxone is given intravenously. If there is a response, a diagnosis of opioid overdose has been established and initial therapy has begun. If there is no response, a second bolus is given to rule out overdose. Immediately thereafter 50 g of dextrose is given intravenously (Strittmatter, 1992). This will reverse the effects of drug-induced hypoglycemia or insulin overdose, or any other cause of hypoglycemia.

In nonhuman primates, as soon as a vein can be located, a bolus of 50% dextrose should be given. Ten milliliters of a 50% dextrose solution should be administered to any primate that weighs more then 5 kg. If the primate is hypoglycemic, there may be an almost instantaneous response. However, because hypoglycemia affects many organs, a response may not be seen for several minutes. It takes a complicated metabolic process to utilize the dextrose. The key organ is the brain which uses only a glucose for fuel. It should be emphasized that brain damage can occur in overzealous treatment of hypothermia because its metabolic requirements are increased by warming. This is one of the reasons a primate may recover from hypothermia only to relapse into unconsciousness due to a lack of sufficient glucose. Therefore, intravenous dextrose should be administered to most comatose primates because it provides three important factors: it is preventative medication in hypothermia, it is diagnostic when there is a response to therapy, and it is treatment for hypoglycemia, regardless of the cause of hypoglycemia.

The risks or side effects of a bolus of dextrose are negligible (Strittmatter, 1992). It will not kill a diabetic primate because the blood glucose levels are already so high. In a nondiabetic primate the induced hyperglycemia is not toxic and the peak level is transient because excess blood glucose is rapidly metabolized and it is also efficiently eliminated in the urine through the kidneys. Probably the most difficult aspect of induced hyperglycemia is caused by its effects on laboratory tests. Before administering the dextrose it is wise to collect a few drops of blood for a quick "dipstick" test (Ames, Elkhart). It is an easy diagnostic test that serves as a baseline for further therapy. It may also identify the rare diabetic primate that is in a diabetic coma. Although dipsticks are easy to use and quite accurate, it is extremely useful to get enough blood for a baseline complete blood count (CBC) and chemistry panel for diagnostic purposes before medications, including dextrose and corticosteroids, are administered.

Ideally, 50% dextrose should be given intravenously. If it is not possible to give an intravenous injection, the dextrose should be administered orally. Because 50% dextrose is quite viscous, it is possible to asphyxiate the patient, especially an infant, when given orally. The 50% dextrose can be dipped on the tongue and oral mucosa, where it will be absorbed fairly quickly if the circulatory system is functioning. Frequently, the circulation is deficient when a monkey is first found. In this situation, the viscous 50% solution should be diluted with water

(tap water is sufficient) to a 20 or 10% solution. It can be given orally if the monkey has good swallowing ability. Otherwise it can be given through a stomach tube or a nasogastric tube. There are definite risks of getting the solution into the lungs if the tube is not properly placed and its location in the esophagus should be verified.

Despite the difficulties that may be encountered, administration of dextrose is a key aspect of the early treatment of a moribund primate. Because of its crucial diagnostic and therapeutic properties, a serious effort should be devoted to its early delivery. If it is not administered, the best resuscitative attempts can fail. Nothing is lost if there is no response to it, but a life may be saved with simple sugar.

4. Trauma: Crush Syndrome

In humans there is a well-known condition called crush syndrome (Stamp, 1984). It was first described during World War II in victims of aerial bombings who were trapped for prolonged periods under collapsed buildings (Bywaters, 1942). Although not obvious, massive soft tissue damage had occurred. This can lead to renal shutdown and death 3–7 days later if not recognized and treated properly. Sometimes the injured were held for observation for 24–48 hr and then released, only to be readmitted semicomatose with greatly elevated creatinine values. The patients finally succumbed to renal failure.

Crush syndrome has killed many primates that could have been saved with aggressive fluid therapy. It is an excellent example of a clinical syndrome that can be cured specifically with fluid therapy.

Creatinine (CRT) and blood urea nitrogen (BUN) can be deceptively low in a severely injured primate with massive soft tissue trauma. The injured primate may suddenly die 2–4 days after it has been traumatized without having a high CRT or BUN. A typical case is an adult female that has been mauled by a group of females. The extent of injury is often underestimated (Norwood, 1992) because there are no major lacerations from canine teeth and external hemorrhage is moderate or nonexistent. The patient may be conscious or apparently dazed. Laboratory values may all be within normal limits. The appropriate treatment may appear to be simple: antimicrobials, steroids, and subcutaneous fluids. The monkey is kept in a warm cage, given soaked biscuits, and a bottle of Tang. It appears to improve for a day or two and is found dead on the third day. Pathology reveals acute renal disease.

C. Iatrogenic Causes

The list of natural causes leading to critical care is almost endless. In contrast, iatrogenic causes are relatively limited. The two major iatrogenic categories are postoperative and experimental. These are not mutually exclusive because postoperative problems may be the result of experimental surgeries. However, postoperative crises that require intervention may be the result of normal life processes such as pregnancies requiring cesarean sections. Experimental causes are those that are the result of specific protocols whose design may create the need for critical care. Unplanned critical care may also be necessary when experimental protocols go awry. Whenever primates are used as experimental models, it is imperative that critical care capability is available.

II. MANAGEMENT

A. Assessment/Biologic Samples

As soon as the core body temperature and blood glucose level have been stabilized, a more thorough assessment of the patient is required. This involves a head to tail physical examination. Rational therapy and patient management depend on an assessment based on physical findings and laboratory data. The physical examination should give the clinician a fairly accurate assessment leading to a plan of action.

Obtaining blood from a downer monkey, especially those weighing under 5 kg, can be very difficult. Emergency treatment should not be delayed in the quest for a substantial amount of blood solely for baseline numbers. A few drops of blood for simple in-house tests can be obtained before or during emergency therapy.

First decide whether the diagnostic cost/benefit reward warrants a pretreatment blood draw. In critical care cases there is a trade-off of which to attempt first, obtaining blood or setting a catheter. Frequently, the best choice is to preserve the largest veins for catheter placement and use smaller veins for blood samples. It is rarely possible to obtain an adequate blood sample using a catheter in a primate in a state of circulatory collapse. If a blood draw is attempted before catheter placement is made, the clinician should make a rapid but thorough survey of available blood vessels. It is always advisable to shave the entire limb length of both arms and legs immediately. This should only take a few seconds. It is imperative to provide maximum exposure of potential vascular access sites.

The large veins of the arms and legs of primates are the first sites to seek vascular access. Terminology varies, but these vessels are most frequently referred to as the cephalic (arm) and saphenous (leg) veins. On large primates such as baboons and apes, vessels crossing the ankle or on the back of the hand may be large enough to be useful. Warming the body or limb of a primate where a vessel is located causes dilation, which greatly enhances the chance of success in entering the vein using a small gauge needle (23- or 25-gauge) or catheter (22- or 24-gauge).

Placement of a catheter is essential for successful fluid therapy. However, obtaining a blood sample for diagnostic purposes is best done with a needle and syringe (3 or 1 ml).

The first priority is to place a catheter; obtaining a pretreatment sample may be a luxury that will not significantly enhance therapeutic decisions in the earliest stages of critical care. However, a great deal of valuable diagnostic data can be obtained from 0.5 ml of whole blood. Even a few drops of blood and/or a capillary tube can be extremely useful using a dipstick and microhematocrit.

The easiest and most important laboratory values that can be estimated are blood urea nitrogen and blood glucose levels. These are measured using dipsticks. Each dipstick requires only one or two drops of whole blood. Within 2 to 3 min a very useful estimation of renal function and blood glucose is available to the clinicians.

A very effective method of rapidly obtaining a few drops of blood is to insert a 22- or 23-gauge needle without a syringe in a peripheral vein. Using this technique, blood can be obtained as it fills the hub of the needle and begins to drip from the hub. The dipstick ideally should be held slightly under the hub so that one or two drops of blood fall onto the top of the dipstick. Instructions for the use of dipsticks are supplied with each bottle of dipsticks and should be read before using them because they must be followed meticulously for this to be a valid laboratory test. The interpretation of dipstick results lies with the clinician. These are simple tests and can give a good indication of the condition of a primate before fluid therapy is instituted and during the course of treatment.

If the BUN dipstick is very dark, it is a sign that aggressive fluid infusion should be started immediately. If the dipstick appears normal, the clinician must not assume that renal function is or will not become a problem if fluids are not administered. A primate that requires critical care usually needs fluid therapy because there are problems that are not directly related to a single estimation of the BUN. The BUN is, as a single test, most useful as confirmation that there are renal problems.

The greatest value of the blood glucose dipstick is to determine if there is severe hypoglycemia. There are many causes of hypoglycemia, but the treatment does not vary.

Assuming that sufficient blood for dipstick tests has been obtained, the clinician must decide whether to attempt a blood draw large enough for multiple laboratory tests, usually a CBC and a chemistry panel. If only a few drops of blood are flowing through the needle hub, two heparinized capillary tubes usually can be filled. Using an on-site, relatively inexpensive centrifuge, the hematocrit or packed cell volume (PCV) can be measured within a few minutes. The reader should refer to basic emergency manuals to find complete information on the use of the hematocrit, its interpretation, and some additional measurements that can be gleaned from the use of a centrifuged capillary tube (Kirk and Bistner, 1990). Usually the hematocrit will be high due to hemoconcentration associated with acute dehydration. However, extremely low PCVs, below 15, have been found in cases of chronic anemia.

If the needle hub has a steady flow with drops of blood "welling up" through it, a fresh sterile syringe should be rapidly but carefully attached. This must be done very gently so that the needle is not pushed through and out of the vein. A 3-ml syringe should be used if possible. A 1-ml syringe can be used, but enough blood cannot be collected from such a small syringe for the desired tests. Before connecting the syringe, it is imperative that the plunger seal has been "cracked" or loosened by moving the plunger within the sterile syringe. A full 3 ml of blood should be sufficient to run a CBC and chemistry panel. If the flow of blood stops, immediately disconnect the syringe and expel unclotted blood first into the EDTA tube to get the CBC. As little as 0.25 to 0.5 ml of good unclotted blood provides a good laboratory with enough blood to perform a CBC. Use the smallest EDTA tube that can be found. Plan ahead and consult your laboratory about their recommended blood volume requirements. Most commercial laboratories provide free sample tubes. If necessary, order the small 1-ml EDTA tubes that are sold. There are now small plastic EDTA "tubelettes" (Beckton-Dickinson, Fullerton, CA) with a flip top cap for micro samples.

As soon as a suitable sample has been collected for a CBC, put as much blood as possible into a red top tube. Although most diagnostic laboratories "require" a full milliliter of serum to run a chemistry panel, one half of this amount will suffice. Try to get 2 ml of whole blood for an essential chemistry panel, but do not waste precious time on getting large quantities of blood. The emergency therapy required at the outset of primate critical care is largely empiric and laboratory results are rarely available in time to significantly alter the immediate treatment.

The needle hub technique described is usually a last resort if a blood sample cannot be obtained by regular venipuncture, but one more modification should be mentioned. If there is a free flow of blood, a butterfly catheter can be connected "backward." That means inserting the hub of the catheter line into the needle hub, preferably using a "male to male" connector. If there is a good flow, a blood sample can be collected less traumatically from the stream of blood exiting from the butterfly needle into the various sample containers.

In addition to venipuncture using a limb, all clinicians and technicians should be familiar with venipuncture at the femoral triangle. This is a more difficult technique, but it is the most frequently used sample site for routine blood draws when more than 3 ml of blood is needed from primates weighing more than 4–8 kg. It is the only practical site when more than 1–2 ml must be obtained from a squirrel monkey or any other small primate in the 0.5- to 3-kg weight range.

As a last resort in an emergency situation where blood cannot be obtained from any of the usual venipuncture sites, a "subclavian stick" could be used. This is a technique adapted from human clinical medicine (Simons and Brenner, 1982). In humans, large long-term catheters are introduced into the subclavian vein. The tip of these large catheters is set a few centimeters above the venous entrance to the heart. The subclavian vein is large and cannot collapse even when there is a general circulatory collapse because it is held open in its normal position by bone and tissue attachments. In nonhuman primates,

routine subclavian catheterization is not possible because of anatomical differences. Except in apes or very large baboons, the chest is too small to present the long subclavian vein. However, in most primates there is a relatively large vein at the junction of the clavicle and the sternum. This vessel, which always remains open, can be a source of a large enough volume of blood for the full spectrum of laboratory tests. A subclavian venipuncture is not an easy technique. A 3- or 5-ml syringe with a 22-gauge needle, 1.25–1.5 inch long is preferable. The length of the needle is the most important component because the vessel is relatively deep in the chest. The clinician's landmark is the notch of the bony junction of the clavicle and the sternum. One finger should be used as the guide while the needle is inserted at a 45° angle through the skin into the chest cavity approximately ¾–1 inch deep. When the vessel is entered there will be a free flow of blood into the syringe. The subclavian stick is difficult and should be the last attempt to obtain blood is an emergency situation.

It should be remembered that the use of a "cutdown" to gain access to a collapsed vein is an effective and widely used technique in human critical care medicine that can be performed in primates with relative ease.

B. Fluid Therapy

1. Introduction

Why and how are fluids used? The answer to the first question is relatively straightforward and the second is unanswerable in a definitive sense. Of all modes of supportive therapy, fluid use is virtually a personal clinical art, even though there are more texts and schemes written than on almost any other treatment regimens. The author has found that there are two references that have the most lucid and useful explanation of theory and practice (DiBartola, 1992a; Rose, 1993). These cover all aspects of the subject, including acid–base and electrolytic imbalances, and diagnostic and therapeutic approaches.

With the exception of diarrhea, fluid therapy almost always begins and ends with parenteral or intravenous fluids. In some cases of diarrhea it is wise to use a combination of intravenous fluids with orogastric fluids when the patient is strong enough to tolerate fluids per os. Subcutaneous fluids can be useful in critical care but are not an adequate substitute for intravenous fluids. They can augment intravenous therapy and will be discussed.

Primates requiring critical care almost always require fluid therapy. Diarrhea causing dehydration (volume depletion) is the most common cause. Trauma victims invariably require fluid therapy to avert or reverse renal shutdown. Primates suffering from severe bacterial or viral infection are usually so debilitated upon presentation that fluids are as essential as antimicrobials. Primates in "shock," whatever the cause, require immediate, intense fluid therapy. Primate critical care depends on a few basic therapeutic modalities to stabilize metabolic imbalance. Fluid therapy is a key component.

There is no precise formula for successful fluid therapy in primates. The existence of numerous calculated formulas for various phases such as correction of base defects (pH), electrolyte imbalances (mEq of Na^+, K^+, Cl^-), and overall fluids (% of body weight) provide essential information, but cannot be used as the sole means to predict, provide, or decide success. Using fluids is in effect a titration of fluid volumes and content against the various monitoring techniques, both measured (laboratory values) and empiric (clinical signs). In primate medicine the majority of patients receive intensive fluid therapy for traumatic and hypovolemic shock. The endpoint or success of fluid therapy in trauma is signaled by a normal heart rate, capillary refill, and, most important, urine output toward normal with decreasing BUN and CRT. In hypovolemic shock due to severe diarrhea or lack of water intake, endpoints would be return to a normal PCV, TP, electrolyte values, and normal vital signs and urine output.

It should be emphasized that successful critical care of a severely traumatized primate depends on the awareness and recognition of the idiosyncracies and details of the crush syndrome. Not every primate with trauma will suffer the crush syndrome, but the potential of this insidious condition supravening must always be considered. Renal shutdown leading to death may not occur until several days after trauma is incurred. Therefore all severely traumatized primates should receive aggressive initial fluid therapy and close monitoring of water intake and renal output. Serial testing of kidney function should be performed for not less than 3 days after the patient is returned to its cage.

Fluid therapy encompasses a vast amount of information that touches on a multitude of medical specialties. It is impossible to cover these areas in depth. Therefore this section presents material that pertains to a typical primate critical care patient that a clinician would encounter on a routine basis. It includes purely technical information and touches specific medical cases and describes clinical issues.

The text is presented in a step by step sequence, which approximates the manner in which a clinical case would be managed. The categories are an attempt to organize the information in a logical format. These are (1) assessment, (2) laboratory data, (3) selection of fluids, (4) monitoring the effect of therapy, (5) outcome, and (6) long-term management. Because there is so much disparate information on fluid therapy, certain topics may be revisited or in odd placement. Before the details of fluid therapy are presented the use of catheters will be discussed.

2. Catheter Placement

To begin effective fluid therapy an indwelling venous catheter must be set in a peripheral vein. Intravenous fluids are the preferred route of administration in most cases. Subcutaneous fluids can be a helpful adjunct, but are not a substitute for intravenous fluids. Oral fluids (PO) have a definite use, especially in longer term care. Administration via intraabdominal or

peritoneal fluids is a crude method and should be avoided other than in exceptional circumstances.

Setting a catheter in primates can often present a formidable challenge because of the small size of many species, in newborns of all species, and the fragility of veins, especially when shock or circulatory collapse occurs. Also, primates generally have fewer access sites than humans and dogs or cats because the jugular veins are not available, except by a cut down.

The cephalic and saphenous veins are the usual sites for catheter placement. In larger primates, veins on the ankle and foot and on the dorsum of the hand may be accessible. Vessels on the tails of some species may be used. In larger males, a penile vein can be used, but this is not a preferred site.

The size, type, and quality of the catheter used are very important. The two types of catheters usually available are the butterfly and the straight indwelling. There are significant variations of both types. It is best to be familiar with and have each available. The most common indwelling catheter used in primates is called an over/around the needle catheter. This type has a thin metal stylus running through the length of the catheter. The other type is a through the needle catheter. These come in sizes that are usually too large (18-gauge and bigger) for use in most primates. The most common butterfly catheter has a short (1 inch) steel needle that is inserted into the vein. The standard attached tubing is 3 and 12 inches.

The decision to use the straight or the butterfly catheter is partly individual preference and partly dependent on the situation. For long-term use (hours to days) the straight catheter is the best choice. It has a relatively long flexible soft catheter that gives it more stability and is far less traumatic to the vessel in which it lies. The term "indwelling catheter" refers to its long-term placement capacity. The short metal tip of the butterfly catheter is much less stable and tends to rip or tear the vessel unless it is perfectly aligned with a flat section of the limb to which it is securely fixed. Even the best placed steel butterfly rubs against the vessel wall and damages the endothelium. To reduce vascular injury there are newer designs that replace the steel tip with a soft indwelling tip (Deseret, Sandy, UT). These butterfly catheters have a thin stylus that goes inside the catheter bore, making it a hybrid around the catheter type. However, replacement of the metal tip with flexible material can significantly degrade the main advantage of the butterfly, which is its ease in placement, especially in small and/or collapsed veins.

The butterfly is most often used as the first catheter that can be successfully introduced in the most difficult vascular access situations. A clinician may choose to use a butterfly on the first attempts or use it as a backup if it is impossible to set an indwelling catheter. When a butterfly is used, generally the best tactic is to use it to get fluid in circulation as fast as possible. As soon as a peripheral vein is "raised" an indwelling catheter should be set.

The butterfly should be retained until steady circulation is restored and there is no question that other indwelling catheters can be placed anywhere as needed. It can also serve as a port for injecting medications, saving the indwelling catheter for fluid infusion only. In a crisis it is always best to have an extra catheter in place in case the main one becomes occluded or is accidentally pulled out of the vein. Also, a second catheter is essential if it is necessary to infuse a large volume of fluid rapidly.

For primate critical care the most frequently used butterfly sizes are 23-, 24-, and 25-gauge with 12-inch tubing. These sizes are small enough to enter most veins and can also deliver a sufficient volume of fluid either to rehydrate very small primates or to raise a vein in larger primates so a larger indwelling catheter can be placed.

The useful sizes of indwelling catheter are 20-, 22-, and 24-gauge. For most uses in primates larger than 5 kg, the 22-gauge is sufficient. In very large primates such as baboons or apes, a 20-gauge is usable and occasionally an 18-gauge may be preferable. A 24-gauge is indicated in infants of most species and in many primates under 5 kg. There is no hard and fast rule which size to use; circumstances dictate size and type.

There are many manufactures and brand names of catheters. In general, the brands used in human medicine are preferable to veterinary brands. The latter tend to be stiffer and inflexible to penetrate the tough skin of dogs and cats. These characteristics are not appropriate for soft primate skin, and they are more traumatic to fragile veins. Probably the most frustrating, time-consuming obstacle to initiating fluid therapy is the difficulty of inserting the first catheter when a vein cannot be found or raised due to circulatory collapse. There is a technique that can overcome this serious problem called "floating the catheter." The vein should be compressed or "held off" a few centimeters above the puncture site. Immediately after getting a good flashback of blood, a 3-ml syringe filled with saline or water should be gently attached to the catheter hub. Then with gentle pressure a small amount of fluid is injected to inflate the vein at the puncture site. Almost simultaneously the catheter is inserted. Continue to inject fluid and insert the catheter. This technique inflates the vein by the pressure of the injected fluid ahead of the catheter. It must be done slowly, a millimeter at time, releasing the pressure on the vein occasionally to allow fluid to flow "up" the vein. It may only be possible to insert one-third to one-half of the catheter at this time. As soon as a free flow of fluid through the catheter is established, as much fluid as possible should be rapidly infused. Then the catheter can be fully inserted. However, the goal is to get enough fluid in so that another catheter can be placed in another vein.

Floating a catheter is a simple, but artful procedure. It saves time and frustration and can turn failure into success. The ability to set a catheter is the technical foundation of fluid therapy.

After an indwelling catheter is secured in place it may be desirable to attach a plastic "cap" (injection cap; Becton-Dickinson, Fullerton, CA) that has a rubber end. There is a very short one (½ inch) that fits onto the catheter hub and is used primarily for the injection of medications. This cap is used to

seal the catheter when it is not being used. After each use it must be flushed and filled with heparinized saline to prevent clotting in the catheter. The short cap can temporarily be used to connect a needle-tipped fluid line. However, when using a fluid line it is best to use a long 1½ inch tapered rubber capped fitting (PRN Adapter by Deseret, Sandy, UT) that is long enough for the entire length of the needle that is connected to the end of the fluid line.

A needle connected to the end of the fluid line is helpful when there are frequent disconnects of fluid lines. It is also much easier and faster to use a needle connection than wrestling with the tight-fitting Luer slip connection of a fluid line directly to the hub of the catheter buried under the tape that is tightly wrapped to the limb. The needle connection also helps prevent inadvertent movement of the catheter that may dislodge it or even pull it out of the vein.

Another advantage of a needle connection is safety. When a primate is returned to its cage with a fluid line and is unattended, it is crucial that if a line becomes disconnected, it does not dislodge the catheter. If there is a disconnect, the PRN adapter prevents accidental hemorrhage that can occur through the end of an open catheter hub. The disadvantage of a needle connection is that it can slip out much easier than the direct Luer lock. This can be a problem during transport of a patient with the attached fluid supply or if a patient is thrashing about. Another slight disadvantage is that some clinicians feel that the volume of fluid flow is decreased through a needle. Usually this can be compensated by using a large bore 18- or 20-gauge needle. If a viscous fluid such as a hyperalimentation solution is being infused, it is recommended that a needle connection not be used.

3. Administration Sets

There are three types of drip sets available to the clinician. These are the standard drip set from which 15 drops equal 1 ml, the micro drip in which 60 drops equal 1 ml, and a set that is referred to as a pediatric or biurette drip set. The first two are relatively inexpensive and are easy to procure. In most cases the standard size, the least expensive, will suffice. The micro drip can be used for virtually all cases, but it costs a bit more. It is recommended for use in small primates when smaller amounts of fluid must be infused slowly and accurately. The biurette set is quite expensive and may be more difficult to procure. It has a cylindrical plastic chamber holding 100–140 ml that is interposed between the fluid bag or bottle and the drip regulator. This allows a set amount of fluid that is let into the calibrated cylinder to be infused without fear of overinfusion from the main source. It is used whenever a precise and limited amount of fluid must be infused, such as when medications (e.g., potassium) are mixed with the fluid source.

4. Assessment

When the patient is presented, a physical examination is performed. The skin between the shoulder blades should be lifted or "tented." Gentle rolling of the skin between the thumb and forefinger is done and then the skin is released and allowed to fall back in place. The texture of the skin and the time it takes to resume its natural formation gives an excellent idea of the state of hydration. This is the most common means of assessing hydration and many references use it to designate the percent dehydration (DiBartola, 1992b). However, species differences and other factors preclude using it as a quantitative measurement. A very important factor that is often overlooked is body flesh condition. A patient that has experienced either abrupt weight loss or has lost a significant amount of weight over weeks or months may appear to be dehydrated when they are normally hydrated. Older primates that lose skin elasticity as a normal part of aging may also appear dehydrated. This may be exacerbated by a gradual weight loss. Despite these confounding factors (weight loss, aging), skin turgidity remains the most common clinical guide to estimate dehydration. Although texts usually try to estimate dehydration on a scale of 5 to 12%, if there is a significant loss of skin turgidity, it is usually best to assume dehydration at 10% in most cases at the outset. This makes rapid calculation of fluid volume replacement easy using the formula % dehydration × body weight (kg) = ml of replacement fluid. For example, a 7.87-kg rhesus that is 10% dehydrated would receive 800 ml. Numbers should be rounded upward; a 13-ml difference is unimportant therapeutically, and precise calculations take valuable time and concentration that is better spent on initiating treatment. The general estimate of 10% is effective and safe in most cases. A 5% dehydrated patient will diurese any excess fluids given on an estimate of 10% dehydration. A limited number of conditions exist where overhydration may be a potential problem, such as congestive heart failure. If a primate is more than 10% dehydrated, it is usually best not to try to correct a 12–15% deficit immediately as this may cause overhydration. Restoration of excess fluids should be accomplished during a 12- to 36-hr period.

Probably the best indicator of hydration is the tongue. This often overlooked barometer reveals an excellent insight and means of monitoring hydration. It is not affected by age or weight loss like the skin. In severe dehydration, the tongue is dry and curled at the edges or it may appear somewhat shriveled. The mucous membranes in the oral cavity are dry and "tacky." During administration of fluids the tongue will gradually resume its normal state and size. The entire oral cavity will become moist and there may be some salivation. It is important that the tongue is not continually exposed to air because it will become dry from evaporation. This may occur if the mouth is kept open, if the patient is intubated. If the mouth has been open, the clinician should moisten the tongue with water or saline. A moistened gauze sponge should be placed on the tongue if it is going to be exposed for a prolonged period of time. The tackiness of the mucous membranes remains a good indicator of hydration.

Observation of the face, especially the eyes, can indicate the state of hydration. Sunken eyeballs may be the first clue of a

problem in singly caged animals. If there is a lot of uneaten food and an animal looks hollow eyed, it almost always signifies trouble. All monkeys will stop eating if they do not maintain water intake. There are many causes of anorexia and it should be noted that monkeys that appear hollow eyed may be normally hydrated. This is because severe weight loss results in diminution of the retro-orbital fat pad, mimicking the signs of dehydration. However, with rapid rehydration, sunken eyes will be restored to their normal appearance if there is not concurrent weight loss.

The triad of doughy skin, "dry tongue," and sunken eyes along with signs of systemic illness are the obvious physical signs of dehydration. Measurement of laboratory values is essential for effective fluid therapy and for diagnosis of the underlying problem. Another important sign of hydration and renal function is micturition. Anuria or oliguria may indicate dehydration from many causes. The first urination is an important sign that fluid therapy may be successful. It is imperative to monitor urine output and quantitate it, if possible.

It should be noted that the term dehydration is not synonymous or accurate for all forms of fluid loss. The body has three fluid compartments: intracellular, extracellular, and intravascular. Each of these compartments reacts differently to various metabolic insults. For example, in shock there are extracellular fluid shifts to help restore plasma volume due to blood loss (Stamp, 1984). This occurs by lowering capillary intravascular pressure, facilitating the movement of fluid from the extracellular compartment into the vascular system. This is a rapid response to shock. At the same time, fluid also moves from the cellular compartment to the extracellular space. This occurs more slowly but the volume is significant.

The term "volume depletion" is used instead of dehydration to more accurately reflect fluid losses from these three compartments. Hemorrhage would be considered intravascular volume depletion. True dehydration would accurately describe fluid losses from all compartments as occurs in a water system failure. However, because dehydration is a familiar, reasonably accurate term and volume depletion is not, dehydration will be used in the discussion of fluid therapy.

5. Laboratory Values

As soon as the first laboratory values are determined, a flow chart to organize the data should be started. Single points of data are usually not diagnostic. Effective fluid therapy is guided by trends because certain values may be affected by factors other than those indicative of dehydration. An example is the PCV. A severely dehydrated primate may have a PCV within normal limits due to chronic anemia. Hematocrits less than 10 have been found in juvenile rhesus that are in relatively good clinical condition (personal communication). A PCV below 10 is the result of a chronic pathology to which the body has gradually adapted. A monkey with this underlying condition may be presented with acute dehydration and a PCV of 20. Therefore it is crucial that the trend associated with fluid replacement is followed. A similar condition may occur in a dehydrated primate that has a "normal" total protein of 8 due to a protein losing glomerulonephritis. Conversely, a mildly dehydrated primate may have a total protein of 14 due to a monoclonal gammanopathy such as multiple myeloma. Therefore both the PCV and total protein should be measured as a cross check to each other and serial values should be obtained.

Usually the PCV and total protein are a good indication of the extent of dehydration (Muir and DiBartola, 1983). These are simple tests that can be performed serially on very small amounts of blood. Elevated values will return toward normal with fluid therapy.

In addition to PCV and total protein, blood urea nitrogen (BUN) can be easily tracked using dipsticks. Partially because the dipstick is an approximation, watching the trend is more useful than the absolute value. Many cases when first presented with severe dehydration may turn the dipstick black going off the scale. This is because of severe prerenal azotemia. If there is no underlying kidney pathology, the BUN will fall quickly and return to normal with vigorous fluid therapy.

A much better idea of renal status is presented by the measurement of creatinine (CRT). This value is reported on the typical chemistry panel, in addition to the BUN. If a clinician can choose only one parameter, the most useful one is creatinine. It is generally considered to reflect more accurately the status of renal function than BUN (Wallach, 1992). It is mandatory to understand that neither the CRT nor the BUN begin to indicate kidney problems until approximately 75% of renal function is lost. On most chemistry panels the BUN to CRT ratio is calculated, with normal being approximately 10:1 (Wallach, 1992). In human medicine, changes in this ratio are associated with various renal conditions and other syndromes, but this information is of little value in primate fluid therapy.

An important question is whether the BUN or CRT is a good measurement of kidney pathology. A more accurate question is whether they are indicators of reversible or irreversible disease. This is impossible to answer in any given patient, but there are some generalizations that are fairly safe. If both values are elevated, especially after baseline and posttreatment values are obtained, the prognosis is not good. If the patient is oliguric or anuric after fluids have been given, kidney function is almost certainly impaired enough to cause death. The most difficult and frustrating situation is when the values are conflicting, especially if they change inversely. The only advice is to continue vigorous fluid therapy, taking special care to prevent pulmonary edema due to overhydration. Probably the most deceptive values are a high initial BUN with a low to moderate CRT in which the BUN falls to near normal with fluid therapy, pointing to a diagnosis of simple prerenal azotemia. This is what is often seen in cases of trauma, particularly soft tissue trauma and crush syndrome.

Blood gases are easier to use and are more available than many clinicians think and can augment fluid therapy use. Easy to use

portable blood gas instruments designed for field use by paramedics are now available (Diametrics Medical, St. Paul, MN). These use disposable cartridges and do not require constant calibration or specially trained laboratory technicians. Obtaining suitable samples can usually be collected without great difficulty. Contrary to common belief, venous samples can provide accurate and useful data. Although preferable, arterial blood is not mandatory because venous blood provides close approximation for most values, especially blood pH, which usually is the most helpful parameter (Shapiro, 1993). Blood gases are also easier to use than is commonly believed because the sample does not have to be immediately rushed to the laboratory. Both arterial and venous samples can be held on crushed ice for at least 1 hr or more before significant degradation occurs. The turnaround time using modern blood gas analyzers is usually less than 3 min, adding to the ease of use. The specific use of blood gas values will be discussed in the context of fluid therapy.

The measurement of blood gases can give the clinician an excellent insight into the metabolic status of a primate requiring critical care. The most common acid–base disturbance is metabolic acidosis. Most trauma patients are acidotic. A great deal of information can be derived from the total CO_2 content of the serum. This value (normal = 24) is usually reported on the chemistry panel. It can also be measured in house on a Harleco apparatus which is a simple and inexpensive technique (Brobst, 1984). A total CO_2 of 12–14 is often found, indicating a metabolic acidosis. Bicarbonate may be added to a fluid bag and infused slowly in a dilute concentration. Severe metabolic acidosis has been observed in humans with bicarbonate losses secondary to diarrhea and may occur in primates. There is a formula for determining the amount of bicarbonate needed to correct an acidotic patient (Brobst, 1984). However, it is easy to overtreat with bicarbonate and primates do tolerate severe acid–base imbalances remarkably well and will reequilibrate with general fluid therapy. The clinician should consult references and have a good grasp of acid–base disturbances before beginning aggressive treatment with bicarbonate.

Measurements of the electrolytes sodium, potassium, and chloride are essential for accurate fluid therapy. These are included in most chemistry panels.

Sodium and potassium are altered in a variety of diseases in primates. Severe diarrhea and trauma are the most frequent conditions a clinician encounters with an electrolyte imbalance. Human and veterinary texts (Johnson, 1992; Rose, 1993) refer to different types of diarrhea that result in specific electrolyte changes. Rarely do primate diarrheas conform to these specific descriptions and a clinician should not expect to guide therapy based on these descriptions. There are formulas that are used to calculate electrolyte changes that can be useful for diagnosis and provide guidance of fluid therapy (DiBartola, 1992b; Rose, 1993). Formulas that calculate the "anion gap" and "osmolal gap" augment the basic electrolyte diagnostic calculations (Feldman and Rosenberg, 1981). If a clinician has access to osmolality testing of serum and urine, use of both gap calculations can be very effective in fluid therapy. The clinician should consult references to understand the details of electrolyte physiology (Rose, 1993). However, a good understanding of the fundamentals of electrolyte changes is sufficient to provide good fluid therapy.

6. Selection

The choice of which fluid to use is probably the subject of greatest dispute in fluid therapy. Any of the commonly used fluids are satisfactory in most cases. The three fluids most used are lactated Ringer's solution, 0.9% physiologic saline, and 5% dextrose in water, often referred to as "D5W." A stock of these three fluids can be useful in the vast majority of cases. Other more specialized solutions are available but are rarely needed. If there is a need for a particular substance, such as potassium, it can be added to any of these three basic fluids.

When the clinician chooses a fluid and begins therapy, a goal and plan of action should be set. A "give it a bag of D5W and see what happens" does not constitute fluid therapy and many cases will worsen or be lost if aggressive treatment is not begun. In critical care a two step approach should be taken. The first is treating a life-threatening crisis and the second is to determine the etiology to find a cure. If one or more indoor-housed primates are found down in the cage with severe dehydration, the cause may be an inadvertent shut down of an automatic water system. Fluid therapy is straightforward and the problem can easily be corrected. A severely dehydrated primate from a corral presents a much more challenging case. Both cases demand a systematic therapeutic approach.

In severe cases of diarrhea there are multiple electrolyte losses. If there is a marked decrease in serum potassium, it may be helpful to use a fluid replacement with added potassium. There are premixed bags of fluids supplemented with potassium. If this is not available, potassium may be added to a bag of 5% dextrose in water. Potassium should never be added directly to an intravenous line due to cardiac toxicity.

The selection of the specific fluid should be based on clinical and laboratory assessment of the patient. The fluid of choice for shock is lactated Ringer's solution (Stamp, 1984) because it provides a balanced electrolyte solution. Two common misconceptions about specific fluids exist. The first is the concept that patients with a lactic acid buildup such as occurs in shock should not be given lactated Ringer's solution because lactate will increase concentrations of lactic acid. There is no clinical evidence that this is a problem (Zaslow, 1984). The second fallacy is that administering 5% dextrose provides a good source of calories. This small quantity of sugar provides minimal energy and in no way should be relied upon as a nutritional substitute. The dextrose is quickly metabolized, resulting in an infusion of free water (DiBartola, 1992b). This unbalanced electrolyte solution may aggravate a decrease in sodium and/or potassium which may occur in diarrhea or if the patient is vomiting.

There are a few situations where a specific fluid is indicated or contraindicated. In Addisons's disease, which is rare in monkeys, saline is the fluid of choice and D5W should not be given because its metabolism to a hypotonic free water base exacerbates a severe lack of sodium and potassium. In acute dehydration, such as in a water system malfunction, 5% dextrose in water would be indicated to restore fluids in all three compartments where sodium and potassium have not been depleted. There are other unusual situations that the clinician is unlikely to encounter where a specific fluid is indicated, but it is hard to do harm using lactated Ringer's solution. In almost all crises it is best to use the fluid that can be started immediately, preferably lactated Ringer's solution. After laboratory results are received, a different fluid can be given, if indicated.

Fluids should be administered that have been warmed to body temperature. This is crucial in hypothermic patients and is very important in small primates that lose body heat rapidly. Primates that are undergoing surgery lose heat and should receive warm fluids.

Several bags of fluid can easily be kept warm in a microbiology incubator. If they are stored at room temperature, they can be warmed under hot running water, submerged in a container of warm water, or rapidly, but very carefully, warmed in a microwave oven. A microwave oven can easily overheat a bag of fluid in less than a minute. Blood warmers are routinely used in human medicine and reconditioned units are available through surgical supply companies. Before administering the fluid the clinician should test its temperature on the back of the hand. It is a good idea for the clinician to personally assess the temperature because inexperienced assistants generally underestimate the temperature, if they only make a quick touch of the bag with a finger. The traditional method to keep fluids warm as they are being infused is to lay one or two coils of the intravenous tubing in a pan of warm water. However, a pan of water cools rapidly, and if the fluids have not been sufficiently prewarmed, a few seconds of contact in a pan of warm water will not significantly increase the temperature. A clear plastic bag that is inflated with warm air and secured around a prewarmed fluid bag can act like a thermopane storm window to keep the fluid bag warm. This may be a bit awkward and its feasibility should be tested before an emergency.

There is always the question of how much fluid should be given over a period of time. After the assessment of dehydration/volume depletion is determined, the total volume of fluid required for repletion is calculated by multiplying the percent dehydration times the body weight. For example, a 12-kg primate that is 10% dehydrated would require 1200 ml. This is only an estimate; clinical response to therapy is the most important guideline. In emergencies, a good rule of thumb is to infuse half of the calculated volume as fast as possible. To expedite an infusion it may be necessary to set two catheters, especially if the first one was a small 24-gauge. The second half should be given over several hours.[1] It is important to realize that fluid therapy should be given to effect, with response to therapy a good measure of efficacy. In addition to the replacement volume that is needed, there is an ongoing need for maintenance fluids. Maintenance is usually considered to be a combination of sensible and insensible loses, estimated to be 10 ml/kg each (total 20 ml/kg) over a 24-hr period.

Subcutaneous (SQ) fluids may be given concurrently with intravenous fluids. If an intravenous catheter cannot be established, SQ fluids may be the only alternative. However, because SQ fluids must be absorbed into the general circulation, a primate in which a catheter cannot be set is unlikely to have the circulatory capacity to effectively absorb SQ fluids. Until circulation is restored the injection site of SQ fluids will remain intact, like saddlebags. Administration of SQ fluids may be painful and no more than 20 to 30 ml should be given at one site. The absolute amount is limited by the size of the patient and by the looseness of the skin, which is anatomically site specific. The best areas are on the dorsal drunk, near the scapulae. To facilitate injections of the maximum amount of fluid and enhance absorption, gentle pressure should be applied to the injection site to disperse the fluid over the greatest possible body surface and enhance absorption. If fluid begins to leak back out of the injection site, no more fluid should be injected. All SQ fluids should be warmed to prevent body heat loss. Oral fluids given with a stomach tube are an option, but the same limitations apply as for SQ fluids. There is also the possibility of vomition and aspiration.

When a patient is returned to its cage and is alert enough to drink, a solution of Tang (Kraft General Foods, Inc., White Plains, NY) should be provided in a bowl or water bottle. A powdered electrolyte mix should be added to the water before the Tang is added. The benefits of oral rehydration were originally demonstrated in the treatment f human cholera patients in developing countries (Hirschorn, 1980). A number of these compounds are available and are inexpensive. If a patient has a low potassium level or has been anorectic, a grape-flavored potassium supplement (Kaon, Adria Columbus, OH) can be added to the mix or to a fresh bottle of water. The oral electrolyte supplement should be continued as long as needed.

7. Monitoring

Patient monitoring must be instituted as soon as fluid therapy is begun. Serial hematocrits should be performed to detect hemodilution. If there is active hemorrhage, fluid therapy can drive the PCV down, especially if a large volume of fluid has been given. A rapid drop of the PCV below 20% may indicate the need for whole blood transfusion. Serial dipstick BUNs are good for following changes in renal function. Certain tests such as BUN and CRT should be performed after apparent recovery to detect long-term sequelae that may not be clinically apparent. As noted, laboratory tests are best interpreted as trends.

[1] A loading dose, given as rapidly as possible, is estimated to be the mean of one blood volume in the dog and cat (90 and 50 ml/kg, respectively).

There is no substitute for clinical signs detected with physical examination. A circulatory overload leading to pulmonary edema is the primary concern in fluid therapy. The chest should be auscultated for moist rales or bubbly sounds. Urine output should be closely monitored. An output of 1 ml/kg/hr indicates satisfactory diuresis (DiBartola, 1992b). Little or no urine output is an ominous sign of impending renal failure. It may require 1–2 hr to obtain the first major urination. Residual urine in the bladder that is passed should not be mistaken for active diuresis. A high specific urine gravity measured with urine dipsticks or a refractometer is an indication that the first urine passed is residual. Once a good diuresis is achieved the urine will tend to have a low specific gravity or osmolality. If urine-specific gravity is isotonic (1.010) after large amounts of fluid are infused, this may indicate a loss of renal function. The kidneys usually lose the ability to concentrate urine before dilutional ability is lost (Wallach, 1978). Therefore urine with a low specific gravity during diuresis is not a sure sign that the kidneys are fully functional.

8. Outcome

If diuresis is not achieved after aggressive fluid therapy, use of a diuretic should be considered. Furesomide is the safest and most effective diuretic. There are no hard and fast rules when or how to use diuretics. Furesomide is relatively nontoxic and if renal failure is suspected, especially after trauma, there is relatively low risk in its use, even in high doses. The author has not had good success with furesomide, but has never encountered any clinical ill effects. Dopamine and dobutamine have diuretic qualities but also have powerful cardiac effects and are difficult to administer. Their effectiveness in primates as diuretics has not been proven. Mannitol, an osmotic diuretic, can have severe side effects and must be used with great care.

In addition to pulmonary edema, overhydration may cause whole body edema, resulting in an overall bloated condition. A subtle but early sign is puffiness of the face. This is especially noticeable on the forebrow. The eyes may protrude noticeably. In extreme body edema the arms and legs may feel slightly crepitus. Expected laboratory abnormalities include a reduction in hematocrit and plasma proteins and an increase in body weight.

9. Long-Term Care

After the acute phase of fluid therapy has passed, consideration must be given to follow-up or "long-term" treatment. The duration of follow-up therapy is variable; it may last for 12–24 hr or may be needed for several days. This is a decision that the clinician must make based on the severity at presentation, etiology, prognosis, and other factors. On a practical basis, ideal therapy is usually circumscribed by the availability and cost of personnel. Very few facilities have the capacity for continuous 24-hr care.

The volume of fluids is based on a deficit or replacement requirement caused by the immediate clinical problem, the maintenance requirement for daily losses (sensible and insensible), and contemporary or ongoing losses such as diarrhea or vomition. Deficits are calculated on the body weight in kilograms times percent dehydration. Most of this will have been replaced in the acute phase. Daily maintenance requirements are estimated at 40–60 ml/kg body weight. If there is or has been blood lost, 3 ml of crystalloid solution should be given for each milliliter of lost blood (Muir and DiBartola, 1983). The normal urine output is 1–2 ml/kg/hr and maintenance fluid must be given to keep pace with urine output. Body weight should be measured twice daily using the same scale. A gain or loss of 1 kg can be considered an excess or deficit of 1000 ml. These figures are only estimated guidelines and should take into account long-term fluid administration or PCV, TP, urine-specific gravity, and, most importantly, the evaluation of the patient's response to therapy.

During long-term fluid therapy, deficiencies or excesses of specific electrolytes are theoretically possible. An anorectic primate with diarrhea may have a potassium deficiency which should be corrected. However, there is rarely a need for intravenous therapy with sodium, potassium, chloride, or bicarbonate. Gross electrolyte abnormalities are most often seen in the acute phase. Even then specific treatment is usually unneeded and overzealous therapy may be harmful. If a primate given lactated Ringer's solution survives a crisis, it will usually reestablish any electrolyte or acid–base imbalance.

III. TRANSFUSION

A. Introduction

Transfusion of whole blood for primates requiring critical care is relatively easy to perform, but is seldom required. Although most primates sustaining large blood losses are those used for experimental surgery or are obstetrical emergencies (placenta previa, placenta abruptio), other conditions can result in hemolytic anemias that require transfusion. Primates presented for severe trauma rarely have significant internal bleeding because there is no deep penetration injury. There is often obvious, sometimes spectacular, bleeding with facial injuries, especially when severe tongue laceration/amputations are incurred. It is not uncommon to find a monkey with up to two-thirds of its tongue traumatically amputated. This frequently happens to primates that fight through chain-link cage dividers. Despite the apparent severity of this unique, hard to understand injury, bleeding usually ceases by the time it is recognized, without clinically significant blood loss. Other common injuries that cause obvious blood loss such as bitten fingers, toes, and tails usually do not have major blood loss.

If a transfusion is indicated due to acute blood loss or chronic anemia, it is best done with fresh whole blood. Although primates have well-defined blood types, these are generally of little clinical significance. Most primates (with the exception of the great apes) of the same species can receive blood from multiple donors during a single transfusion without incident. In the rare instances where a primate has had a transfusion in the past, they do not suffer any clinically apparent reaction to subsequent transfusions. However, some facilities perform cross matches for research purposes. The major cross match tests erythrocytes of the donor against serum of the recipient, and the minor cross match tests the serum of the donor against erythrocytes of the recipient. Incompatibilities in the major cross match are considered the most significant clinically.

B. Collecting Blood

The basic whole blood transfusion is simple and direct: a donor is selected and immobilized, blood is withdrawn into a container, and is then immediately infused into the recipient. Any healthy adult is a potential donor. However, some facilities have designated blood donors. These donors are screened to ensure that they are free of certain viral antibodies such as simian immunodeficiency virus. Donors receive daily multivitamins with iron and their hematological status is checked once a month.

Generally the largest adults, usually males that are close at hand, become donors. The most critical technical aspect is providing a container with the proper amount and type of anticoagulent. Anticoagulants that may be used include heparin (1000 units/ml), acid–citrate–dextrose (ACD), citrate–phosphate–dextrose (CPD), and citrate–phosphate–dextrose–adenine (CPDA). The latter two (CPD and CPDA) afford longer shelf-life if blood is being stored. Clinically, neonatologists prefer heparin over ACD because ACD may change the pH in neonates and infants. Heparinized blood (100 units to 10 mls whole blood) should be used immediately. Ideally, blood should be collected in plastic containers. The standard plastic bags used for collecting human blood come in two sizes, each of which has enough ACD or CPD to collect 250 or 500 ml of blood. The 250-ml bag is preferable and will accommodate enough blood for the largest primates. Rarely is even 250 ml collected at one time. Therefore ACD, which is the anticoagulant commonly available to primate clinicians, has to be removed from a bag proportionate to the amount anticipated to be collected. A ratio of 1:6 (one part anticoagulant to 5 parts blood volume) is satisfactory (O'Rourke, 1983).

Two techniques are used for the actual blood withdrawal in primates. The first is direct collection into a large syringe (30 or 60 ml) containing anticoagulant. Usually a femoral venipuncture is used. In primates weighing over 20 kg it may be feasible to collect from a large peripheral vein. When drawing blood the anticoagulant should be thoroughly mixed with the donor blood. This may be difficult to accomplish with a syringe unless the blood flows rapidly into the syringe. Passive gravitational flow into a plastic bag is slower, but the contents of the bag can be gently, but frequently, mixed. A simple gravity flow can be accomplished using a 20-gauge butterfly needle with 12-inch tubing. A femoral venipuncture is performed with the butterfly and the blood is allowed to drip into the plastic bag. This requires two persons and should be practiced before the actual need arises. The butterfly technique is equally useful when blood is collected in a large syringe.

C. Administration

The blood should be administered through an indwelling catheter. A blood administration set that includes a filter (venoset) should always be used. Direct injection from a syringe of whole blood without a Millipore filter is not recommended. A rule of thumb for the rate of administration is 10 ml/kg body weight per hour. Faster rates may be necessary in life-threatening situations. Response to therapy and clinical judgement should supersede any particular formula.

The total amount of blood given is determined by clinical status, the underlying problem, and response to therapy. An estimate of this amount needed can be determined based on the PCV and weight of the recipient, the PCV of the donor blood with anticoagulant, and the lowest acceptable post-therapy PCV. Normal blood volume is estimated to be 80 ml/kg (Stone and Cotter, 1992).

IV. WOUND MANAGEMENT

A. Introduction

The best critical care that is provided to primates in the acute phase, such as fluid therapy, may be of little value if the underlying problems are not recognized and attended to properly. Traumatic, massive soft tissue injury that causes the crush syndrome may lead to infection, overwhelming sepsis, and death. As soon as aggressive fluid therapy has been started and the patient is stabilized, wound management must be performed and provided concurrently with supportive therapy.

One of the most important concepts in treating wounds is to realize that they are dynamic. Treatment usually must be modified based on the progression of the wound and surrounding tissue involvement. Soft tissue wounds are like an iceberg; only the outermost layer of injured tissue is usually visible. Most of the physiologic damage is hidden and more damage develops during treatment. Frequent assessments and appropriate therapeutic modifications are required for successful wound healing.

There are many texts that review typical wound managements. Because of the unique injuries that primates suffer, specialized techniques are required to successfully treat those injuries. They are empirical modifications of wound manage-

ment regimens that most clinicians have not been exposed to in school or postgraduate training. These techniques are wet to dry and tie over dressings. Skin grafts may be used after the wounds have been prepared by using these dressings.

B. Wet to Dry Dressings

Wet to dry dressings are classified as adherent dressings. They are designed to protect soft tissue wounds and most importantly are used to remove necrotic tissue (debridement) and control infection that may lead to sepsis and renal failure.

The components of wet to dry dressings are simple and inexpensive. These are rolls of 1- and 2-inch fine mesh gauze (hard gauze) and wide mesh gauze (Kling, Johnson & Johnson, Arlington, TX), white adhesive tape, saline, sodium hypochlorite (plain laundry bleach), povidone–iodine (Betadine solution, not scrub), and a pair of scissors.

1. Assessment

Aggressive local wound management begins with wound assessment. Lacerations, freely bleeding injuries, and fractures are obvious and can be dealt with relatively quickly and are basically one time treatments. Soft tissues damage may be difficult to assess at presentation. Even more difficult is estimating damage that is not clinically apparent but develops during hospitalization.

2. Application

Wet to dry dressings may be applied anywhere. Most commonly they are used, with greatest ease, on wounds on the limbs of primates. Before application it is essential that all visible necrotic tissue has been excised. Wet to dry dressings are useless if a wound contains large amounts of dead and dying tissue. Aggressive debridement must be completed or else the wound will suppurate under the dressing. After debridement the fine mesh gauze is applied directly to the wound. This may be done by wrapping shallow wounds with several layers. Deep wounds should be packed with strips of gauze and then wrapped. Ideally the first layer of gauze will adhere to the moist surface tissue by itself. If this does not occur the first two layers of gauze should be moistened with an antimicrobical solution to ensure adherence.

After the hard gauze has been applied it should be fully moistened, but not soaked with the solution of choice. The wide mesh gauze (Kling) should be wrapped over the fine gauze. The bulk of the dressing is made of Kling. There should be two to three times the amount of Kling to hard gauze. The dressing is then secured with adhesive tape, preferably only one to two layers of tape directly over the wound. However, the dressing must be kept securely in place. An elastic wrap (Vet Wrap, 3M, St. Paul, MN) may cover the entire dressing. If Vet Wrap is used the clinician should make sure that it is not too tight, impeding circulation to the site. Frequently overzealous, improperly applied Vet Wrap has cut off circulation to many bandaged areas which causes a disaster and in extreme cases has lead to amputation.

A wet to dry dressing may be left in place overnight, but in severe cases it may have to be changed two to three times in 1 day. To be effective the hard gauze should be completely dry when removed. Success is evident when necrotic tissue is adherent and/or absorbed into the gauze. In severe cases the gauze will be molded to the wound surface and may literally break off the wound site. If the gauze is still wet at removal, it cannot do its job properly.

When used aggressively but with care, wet to dry dressings may change intractable, nonhealing wounds into clean, rapidly healing tissue within a few days. Wet to dry dressings can produce almost miraculous results, transforming gaping wounds into bright red granulating tissue that can fill in and heal by contraction with no additional therapy; they can prevent serious, acute wounds from becoming septic, potentially life-threatening injuries; and they can be used to repair a bed of granulation tissue for skin grafts.

The choice of solution depends on the stage of the wound surface. Initial dressings should use the Dakin's solution (12 mls bleach, 6 grams sodium bicarbonate, 88 mls sterile water) for maximum debridement. Dakin's solution liquefies necrotic tissue and kills bacteria. However, it may delay the growth of new tissue and slow the healing process. Betadine solution may be substituted for Dakin's solution when granulation tissue first appears. When the need for antimicrobial activity is finished, plain saline should be used. Which solution to employ should become evident if the dressings are being used to good effect.

C. Tie Over Dressings

Tie over dressings are actually a method to secure a wet to dry dressing (or other bandage) in difficult sites on the body that are not amenable to adhesive tape. They are basically a bundle of material tied with strings.

A typical site for a tie over dressing is on the top of the skull where a head implant or pedestal has been removed. Six or eight silk or Vicryl (Ethicon, Somerville, NJ) sutures are placed in the skin around the dressing site. One end of the suture is secured in the skin and a length of the suture is left uncut, usually a few centimeters long. A wet to dry dressing is made with the two types of gauze. No adhesive tape is used. The dressing is placed on the wound site in the manner it would be done on any wound. Suture strands that are opposite each are tied together tightly enough to exert downward pressure on the dressing. This is repeated with the remainder of the preplaced sutures. When finished the primate has what looks like a bundle or top knot tied to its head. The same principle of removing and replacing a wet to dry dressing is continued until the wound has a good bed of granulation tissue.

It is expected that there will be several dressing changes, requiring preplaced circular anchor sutures around the wound site. The dressing is held in place by running a suture through opposing anchor loops and tying the ends together over the dressing to hold it in place. When the dressing is changed, only the tiedown sutures are cut to remove the dressing and the anchor loops are reused. This saves a great deal of time compared to putting in complete tie over sutures. Anchor sutures may be made with monofilament suture material to reduce inflammation and reduce contamination that may occur with braided sutures.

Vicryl or silk is used for the tiedown strands because these materials tie to each other better and are easier to handle.

D. Protective Dressings

Primates are experts at picking sutures and pulling off the best of tiedown or taped dressings. An effective means of prevention is to bandage their hands and/or feet with bulky bandages that look somewhat like "boxing gloves." These bulk bandages are effective in most primates. If they chew on something, they usually direct their attention to immobilized hands and leave the wound dressings alone.

These gloves are made of roll cotton, Kling, and tape. They are sometimes referred to as Robert–Jones splints. To make a safe but effective glove takes practice. The basic strategy is to envelope the hand or foot with cotton and tape over it. Each clinician has to experiment with their own particular method. A brief description follows.

The limb is shaved to the mid-forearm and three to five strips of 1-inch adhesive tape are applied longitudually from the wrist to midlimb to create a good surface for final taping. Tufts (strips) of roll cotton are laid between the fingers or toes that extend from the dorsum to the palmar surface. These tufts are laid between the digits to protect them from compression of the overlying bandaging. A tuft may also be placed in the palm itself. A half-thickness of roll cotton is then wrapped around the entire hand. It is crucial that enough material is in place to prevent compression and cut off of blood supply to any portion of the hand. Kling is then wrapped around the bulk cotton, crisscrossing the hand and going between each finger. Sufficient Kling is used to make a complete layer over the cotton. The combination of cotton and Kling encases the hand so that it is buried and immobilized. Some clinicians allow the tips of the fingers to protrude in order to help detect swelling due to compression. The Kling is continued past the cotton and up the arm over the extent of the first strips of adhesive tape. One- or 2-inch adhesive tape is then used to cover the entire surface area of the bulk material. This completes the glove. A few winds of elastic Vet Wrap may be used to cover the tape. Elastic wrap should *never* be used as the primary covering over the Kling because it frequently causes compression problems. If an outer layer of Vet Wrap is used, precaution should be taken so that it does not cover the end of the bandage at the midlimb site because even a single layer may cause compression where it is applied directly to the skin.

The gloves, if properly applied, can be left in place for several days while wet to dry dressings are used. The clinician should be vigilant to detect any sign of swelling or skin discoloration. To prevent or reduce swelling, two or three longitudinal cuts can be made at the junction of the skin and bandage at the midlimb.

If gloves are used, provisions must be made so that the patient can eat and drink. A metal grill should be placed in the cage over the bars so that food can be placed on it so that the monkey can pick up biscuits with its mouth from the grill or from metal food racks. The grill must be cleaned daily to prevent fecal buildup. The clinician must make sure that the monkey can get ample food and fluids if its prehensive ability is blocked by bandaging.

E. Skin Grafts

The combination of wet to dry dressings used properly with tie overs and glove restraints will hasten and complete healing of most wounds. The more necrotic and gaping a wound appears, the more remarkable results will be using wet to dry dressing in an aggressive, proper manner. However, some wounds, due to position or extent, may not be able to be closed with delayed suturing or may not heal by filling in with granulation tissue and contraction of wound edges. A split thickness skin graft may be applicable in this situation.

There are many types of skin grafts, but a simple split thickness autologous graft is the most practical graft available to most primate clinicians. This is a relatively easy techniques that can be done in primate medicine.

The single special instrument required is a Weck (Research Triangle Park, NC) dermatome, which is relatively inexpensive and is available by order through most surgical supply stores. This instrument is basically a fine-edged, controlled-depth straight razor.

The wound to be grafted must have an excellent, sparkling red bed of granulation tissue. No graft will be successful if the site is not properly prepared using debridement and wet to dry dressings.

The best site for obtaining autologous split thickness graft tissue is the external (outside) thigh. This provides a large smooth area that will rapidly heal after the graft is transplanted.

The thigh should be surgically prepared as for a routine procedure. However, after it is "prepped" with Betadine solution, dilute alcohol should be used as the final prep to remove the Betadine which tends to make the site "sticky" and more resistant to the Weck dermatome. A new blade should always be used for each graft that is harvested.

The skin is harvested using the dermatome in a downward back and forth motion to obtain the largest graft harvest. The

motion is best characterized as slicing a side of beef, albeit in a very fine smooth degree. The graft tissue is placed on a moistened saline gauze pad and is immediately laid on the wound. The objective is to cover as much of the wound site with a single piece of tissue. Frequently one section that is large enough is not obtained. Two or more sections should be cut and fitted to the wound edges, like a jigsaw puzzle. Sections of the tissue that overlay the wound edge will necrose and die if they are not in direct contact with the bed of granulation tissue. It is more useful to trim edges and use pieces to fill in the wound site. A graft may be very successful if there are enough "islands" of tissue scattered over the wound site because all edges of tissue will grow out to each other and mold to eventually cover the entire wound. A graft may be successful with only 50–60% of the site covered if the granulation bed is sparkling red and the tissue is harvested correctly. Primates have incredible healing attributes if the wound is clean and good granulation tissue is present.

The grafted site may be covered with a layer of petroleum-impregnated fine mesh gauze (Xeroforme, Baxter, Deerfield, IL) for protection. A graft site, especially on the head, may be left uncovered which is the best biological approach because any dressing material tends to interfere with the healing process. Rhesus monkeys often do not pick at graft sites on the head. A skin graft is a soothing natural protection that greatly increases comfort.

The donor site on the thigh will have only capillary bleeding that may be controlled with mild compression with a gauze pad. When the bleeding has decreased, Xeroforme or Scarlet Red gauze may be applied. After recovery from immobilization with ketamine or Telazol (Telazol, Fort Dodge, IA), the gauze will fall off or be picked off by the monkey. The donor sites do not become infected and heal rapidly. After recovery, analgesia may be indicated, mainly for the sedative effective so that the monkey is quiet and does not rub off the graft. Oxymorphone at 1–2 ml of a 1.5-mg/ml solution is indicated depending on the weight and condition of the monkey.

Split thickness skin grafting is a relatively sophisticated level of care in primate medicine. However, it is basically a simple, inexpensive procedure. The clinician should practice on cadavers, preferably fresh ones so that the feel of the tissue approximates a clinical case. A better learning experience can be performed on a primate that is being euthanatized, when tissue harvesting is the same as in clinical conditions.

V. NUTRITION

A. Introduction

Nutrition during post acute fluid therapy is important, but is frequently overlooked or mismanaged. All animals have a basic caloric requirement which must be fulfilled. Primates receiving critical care may be malnourished, usually due to anorexia. Meeting caloric requirements is best accomplished enterally. However, during prolonged fluid therapy or when treating cachectic primates, caloric intake to a certain extent may be provided by vein. This is based on an average daily requirement of 50 cal/kg/day. A common error is to rely on the frequently used 5% dextrose in water solution. Many clinicians give D5W as their primary fluid, believing that this fluid does "double duty" in replacing fluid volume and providing a good energy source. However, 5% dextrose provides a miniscule 0.2 cal/ml. This does not supply even a small fraction of the daily caloric requirements.

B. High Energy Fluids

A substantial portion of short-term caloric requirements can be provided using a high-energy fluid solution composed primarily of dietary fats or lipids. These are viscous, milky-looking solutions that provide 1–2 cal/ml, depending on which strength product is used. These solutions have been developed within the last 10–12 years and are available under various trade names. Intralipid (Baxter, Deerfield, IL) was the first of these products and originally provided 1 cal/ml. It is now available as a double-strength solution that provides 2 cal/ml. A lipid solution should be employed after the patient is stabilized and on a steady infusion. A bottle of lipids can be infused into the same line that carries the crystalloid fluid. However, because of its viscosity, it may back up the primary fluid line. It is best to infuse it through a separate line, using a 20-gauge catheter. There are limits to the volume used over a set period of time as described by the manufacturer. In clinical use, these limits have been exceeded substantially, with no ill effects. However, at high rates, blood samples that are drawn will look milky white, which is a sign that the rate of infusion should be slowed. Bottles of lipid solution come with their own special drip set that is to be discarded with partially used bottles. However, lipid solutions are expensive and partially used bottles may be safely refrigerated for later use. Also, standard drip sets may be used in place of the special drip set that come with the bottles.

C. Long-Term Management

The use of lipid solutions is only a short term, stop gap approach to meeting nutritional needs. In human medicine, total parenteral nutrition is routinely used. This is impractical in primate medicine because these solutions are hyperosmotic and cause phlebitis if not infused through a large vein such as the jugular. Total parenteral nutrition is also expensive, time-consuming, and requires close monitoring of laboratory values. If nutrition is an important factor during critical care, the most efficient method is tube feeding. This may be accomplished with an orogastric or nasogastric tube. If a primate is given fluids over several days, tube feeding is best done after the

fluids are administered, before the animal is returned to its cage. Nutrition is an important part of primate medicine and must be addressed as a separate issue from critical care. However, it is important that the clinician realizes that meeting caloric needs is part of the overall treatment of any ill primate, this can be partially provided for using certain products as an adjunct to fluid therapy.

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CHAPTER 14

Breeding

Andrew G. Hendrickx and W. Richard Dukelow

I. INTRODUCTION

Effective maintenance of a breeding colony of nonhuman primates is based on a number of factors. The first of these is a basic knowledge of the reproductive physiology of the species involved. This subject has been treated extensively in Chapter 9. Unfortunately, the status of nonhuman primate research is such that there are often large gaps in breeding knowledge, particularly in the species that are not normally held in captivity or are heavily involved in research programs. For the effective breeding of these animals, one must rely on reports from zoological gardens and private or commercial colonies. Additionally, field studies of the animals activities in the wild are very useful in establishing breeding colonies. The final factor is often the common sense husbandry that individuals acquire through work with a large variety of animals in captive situations. This combination of sources of knowledge will become evident in

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this chapter as we deal with Old World species and a few of the New World species, where a great deal is known of their reproductive biology and breeding behavior. We will also examine the great apes, the prosimians, and some other less well-known species where the knowledge is incomplete and a combination of the factors just mentioned must be applied. It must be emphasized that this chapter does not present a comprehensive bibliography; rather, an attempt was made to include those references which best illustrate the breadth of knowledge that has been accumulated on breeding nonhuman primates under a variety of conditions.

II. BREEDING COLONIES

In an attempt to produce self-sustaining primate populations, a variety of species have been bred in domestic and international facilities using four different methods: free-ranging island colonies, semi-free-ranging corral colonies, pen or run-type single-male harem colonies, and colonies where animals are cage mated in pairs. A summary of domestic breeding production of more than 30 species of nonhuman primates has been provided by Johnsen and Whitehair (1986). Mortality and survival rates of colony-born infants of New and Old World species at one of these facilities, the New England Regional Primate Research Center, have been summarized by Johnson *et al.* (1986). Chapter 9 contains information on both cycle and gestational lengths for a number of prosimian, New and Old World species, and great apes. These data are not repeated here; readers are referred to Chapter 9 for this information.

A. Prosimians and New World Species

Information on the seasonal breeding or birth patterns in nonhuman primates should be reviewed with caution, particularly regarding the prosimians. In the wild or seminatural environment, nonhuman primates assume natural seasonality characteristics that are specific for the species (Lindberg, 1987). However, the stresses of captive and/or controlled environments and relocation to varying latitudes (on occasion opposing) result in a period of adjustment until new seasonal patterns are established. Thus, when reviewing the literature for a given species, consideration of the location and housing conditions becomes essential. Accordingly, in this chapter, breeding or birth seasons are reported for colonies located in the northern hemisphere only. These data must also be viewed with some caution regarding the location of the laboratory and environmental conditions.

The total overall effect of seasonality on reproduction in the prosimians has been extensively reviewed (Pollock, 1986). As with most prosimians, the management of galagos varies and is based on the experience of the colony and previous housing conditions for other nonhuman primates. At the Duke University Primate Center, the galago colony is housed indoors with a light:dark cycle of 12:12 hr (lights off at 3:00 p.m.). Both the lesser (*Galago senegalensis moholi*) and greater (*G. crassicaudatus*) galago colonies are housed in pairs or trios (one male, two females) in cages of vinyl-coated wire. Males are removed from cages housing the pregnant females shortly prior to delivery. The male is reintroduced to the female and her young several months later. In one colony of lesser galagos, alteration of the light cycle from 12:12 to 14:10 resulted in normal cyclicity with no evidence of seasonality (Darney and Franklin, 1982). It was found that ovulation occurred from the afternoon of Cycle Day 2 to the morning of Day 3.

A colony of greater galagos was maintained in Davis, California, under conditions of individual or paired housing and a 12:12 hr light:dark cycle. The first conception occurred between 15 and 18 months of age with an average of 1.2 conceptions per female per year; no postpartum estrus was observed. Reproductive capability decreased in the colony between 10 and 12 years of age (Hendrickx and Newman, 1978). In contrast, observations of a colony of 36 animals maintained in the Washington, D.C., area indicated the occurrence of a postpartum estrus; 81% of these conceptions resulted in live births compared to 68% during regular estrus conceptions (Valerio *et al.*, 1972). Others report a 50% conception rate, which declines after 6 to 8 years (Eaglen and Simons, 1980).

Few pottos (*Perodicticus potto*) have been born in captivity and there are even fewer reports of their being successfully reared. There is a single report of an offspring born 219 days after pairing (Blackwell and Menzies, 1968). The slender loris (*Loris tardigradus malabaricus*) has been reared in captivity with females housed with younger offspring in a social group with males introduced for breeding purposes only. Generally, these animals were housed under a 12:12 hr light:dark photoperiod. Under these conditions, single offspring were born at an interbirth interval of 9.5 months. The reproductive rate of the slender loris is among the lowest of any primate weighing less than 500 g (Izard and Rasmussen, 1985); reports for the slow loris (*Nyticebus coucang*) indicate 19 infants (all singletons) born over a 10-year period at Duke University. A postpartum estrus was observed in three individuals but no conceptions resulted. Females were housed as pairs with adult males and the most recent offspring were kept in the social groups until weaning. An effort was made to provide an artificial photoperiod set for the latitude of Malaysia, but this was eventually changed because of space limitations (Izard *et al.*, 1988).

The best evidence for photoperiod regulation of the cycle exists among the lemurs. This has been well-documented in the ring-tailed lemur (*Lemur catta*) (Van Horn, 1975; Van Horn and Resko, 1977). After photoperiodic activation, most impregnated females failed to resume estrous cycles even after infant separation unless they received additional photoperiod changes. Rasmussen (1985) conducted a comparative study of breeding seasonality and litter size in 11 taxa of captive lemurs. Data demonstrate a photoperiodic cue which initiates reproductive

activity preceding the actual mating season by about 2 months with an intervening period of physiological and social preparation. Despite the photoperiodic control, evidence exists in a number of species, such as the mouse lemur (*Microcebus murinus*), that alterations in the seasonal sexual cycle are due to pheromone-like effects among males (Schilling *et al.*, 1984).

The ruffed lemurs appear to be the most resistant to adverse effects of capacity among the prosimians. Litter sizes are normally two to three and the animals are raised in a wide variety of environmental conditions, including standard lighting which is adapted to the local sunrise and sunset. The two major colonies in the United States are at Duke University and the San Diego Zoo, whereas in Europe, the Basal and Cologne zoos have large captive collections (Boskoff, 1977). At the San Diego zoo, breeding pairs are housed in interconnected outdoor enclosures. The success of breeding colonies has been demonstrated by the fact that the San Diego zoo reported 230 ruffed lemurs born between 1969 and 1985. Females will experience up to three cycles during breeding (30–40 days apart) and pregnancies can occur in any of the three cycles, although the majority conceive during the first cycle. Specific breeding behaviors occur only during the reproductive cycle for both the male and female. This includes male aggression toward conspecifics and keepers, chest rubbing, hyperactivity, and squealing. Females also show an increase in aggressive behavior directed toward the adult males and vaginal rubbing (Brockman *et al.*, 1987a,b; Shideler and Lindberg, 1982).

In contrast to the ruffed lemur, reproduction in the mongoose lemur (*Lemur mongoz*) is exceedingly poor. In this species, reproduction has occurred initially with wild-caught specimens but rarely occurs in the captive F_1 generation. Production among 20 institutions shows extreme reproductive failure (Schaaf and Stuart, 1983).

The squirrel monkey is widely distributed in Central and South America and is the third most frequently used nonhuman primate in research. In their native environment these animals are diurnal and arboreal, living in troops of up to 80 animals high in the forest canopy. Even though arboreal, they do spend considerable time on the ground, especially during the breeding season. The first captive births were in the early 1940s, but it has only been since the mid-1970s that emphasis has been placed on breeding in captivity. The reproduction and breeding of the squirrel monkey have been reviewed (Dukelow, 1983, 1985). It is assumed that colony-born squirrel monkeys will eventually be proven to be more productive than wild-caught animals. Initial studies indicated that this may not be the case as the stress of captivity often results in adverse reproductive effects. With increased awareness of the requirements for breeding in captivity, more animals are being produced from captive-born animals. The production colony of the National Institutes of Health breeding program at Mobile, Alabama, is an excellent example.

An annual conception rate of 50–70% can be expected after squirrel monkeys have adapted to the captive environment, but this must be considered in relation to a high incidence of stillbirths or abortions (16.8%) in addition to neonatal deaths (34.3%). A number of laboratories have reported management procedures for the breeding of this species with varying degrees of success. Females and males are normally housed at a ratio of 10:1 in large group cages or in smaller cages at a 3:1 ratio. Male squirrel monkeys are tolerant of the females throughout gestation and birth, therefore it is not necessary to remove males prior to parturition. Single housing of squirrel monkeys should be avoided as the social requirements of the animal must be met for health and survival. A controlled light:dark cycle does not appear to be a strict requirement for normal reproduction. One interesting attempt was undertaken to evaluate the fertility of captive squirrel monkeys which employed continuous mating procedures coupled with daily swabbings to determine when mating occurred (Stolzenberg *et al.*, 1979). Using this technique, 24% of the animals mated and 46 (of 277) full-term progeny were produced. With continuous cohabitation, the mean time from male introduction to insemination was 5 days with 75% of the matings occurring within 8 days.

A number of very successful marmoset (*Callithrix jacchus*) colonies have been established with a high productivity (2.5–3.3 offspring per pair per year) and a relatively short interbirth interval of 154–158 days (Box and Hubrecht, 1987; Poole and Evans, 1982). However, some investigators have noted that high rates of abortion, premature delivery, and early neonatal deaths are common in captive marmosets (Hampton *et al.*, 1966; Phillips, 1976; Poole and Evans, 1982). An account of breeding techniques for marmosets has been published (Hearn, 1983). Because of their small size, marmosets are usually maintained in family groups in captivity but, as with the tamarins, there is suppression of the younger females by the presence of the dominant female. If the dominant female is removed, then the next female in the hierarchy will begin to cycle. Because of this feature, it is practical in laboratory situations to maintain breeders as families and remove the offspring after they have participated in the care of their younger siblings. It is also advisable to house the animals in cages with isolation from neighboring groups (Epple, 1978) since reproductive capacity is maximized with this type of management system. Ironically, marmosets breed better in captivity than they do in the wild, where groups consist of a dominant, monogamous breeding pair, the dependent offspring, and separate hierarchies of subdominant males and females. Under these natural conditions, only the dominant female in a group will breed.

Although successful colonies exist in the United States, the marmoset has achieved a degree of prominence among British laboratories; early work at the ICI laboratories by the late Dr. Bill Hiddleston provided the basic information for reproduction in captivity. Despite the high abortion and deaths, it is possible to maintain healthy and growing colonies of this species. Postpartum ovulation occurs in marmosets and lactation does not interfere with follicular growth and ovulation (Kholkute, 1984) (see Chapter 9). Seventy-four percent of the animals ovulate

within 21 days postpartum and the mean time of ovulation has been reported to be 10.5 days after birth. This feature adds greatly to the fecundity of marmosets. Females will raise twins and triplets at 4.5-month intervals over several years.

A colony of indoor-housed marmosets in Germany maintains permanent breeding pairs in single cages with young animals (approximately 3 months of age) in large gang cages containing 25–35 males and females until they reach maturity (Heger and Neubert, 1988). The success of these conditions has been demonstrated by a gradual shift of litter size from singletons and twins to twins, triplets, and quadruplets over a 10-year period since establishment of the colony. A direct relationship between light intensity and breeding success (i.e., lower breeding rates in darker rooms and/or cages) was also demonstrated in this colony.

The tamarins (*Saguinus* spp.) also have dominant female suppression but the animals are not as productive as the marmoset. The suppression of sociosexual behavior can be measured endocrinologically by the patterns of estrone and estradiol secretion in the urine (French *et al.*, 1984). The twinning rate among subspecies ranges from 1.8 to 2.6 live births per pair per year, at least in the early stages of captivity (Wolfe *et al.*, 1975). The interbirth interval in tamarins is 12 months with animals that rear live young, and averages 7 months for those whose young die prematurely (Kirkwood *et al.*, 1983; see Table I). The cotton-top tamarin (*Saguinus oedipus*) has a low reproductive rate in captivity. As with the marmosets, most tamarins are raised in pairs or small family groups. Price and McGrew (1990) studied cotton-topped tamarins maintained in groups with size and age–sex composition which were similar to those of wild groups. Based on 6.5 years of records from the breeding colony, reproduction was similar to other colonies. Survival was the highest recorded for the species with 60% of the infants reared by their parents to adulthood with the mean surviving litter size of 1.5 infants. Abortion, stillbirth, and parental neglect were rare. Thus, it would appear that the improvements in husbandry technology for tamarins have developed along similar lines as those described for marmosets and squirrel monkeys.

The basic reproductive physiology and breeding of the owl monkey has been summarized (Dixson, 1983). The animals are housed with one or two females per male, each of the same karyotype. Under these conditions, births occur throughout the year. In one colony, a 26% birth rate was attained during the first 9 months. Although twins are not common, they do occur. The mean interbirth interval for owl monkeys appears to be 253 days. There is no postpartum estrus in this species.

Capuchin monkeys (*Cebus apella*) are normally kept in harem groups of one male to five females housed either in compounds or cages (Nagle and Denari, 1982). Reproduction in this species has been summarized by Nagle and Denari (1983). Most wild-caught capuchins are juveniles and it is necessary to identify animals of reproductive age for breeding purposes. This can be estimated by the stage of eruption of the canine teeth and by approximate body weight (2.5 kg for males and 1.8 kg for females). In contrast to nocturnal breeding under laboratory conditions, copulation will occur at any time during the day if the animals are housed outdoors (Nagle and Denari, 1983).

Considerably less work has been done on developing breeding colonies among other New World species. Fontaine and Hench (1982) reported attempts at breeding howler monkeys (*Alouatta* spp.) at the Monkey Jungle in Miami, Florida. Four animals developed a successful breeding unit and produced a single young each year for 3 years. Shoemaker (1982) reported on seven female black howler monkeys (*Alouatta caraya*) held in a zoological park. Twenty-three births occurred from this group but little is known regarding the basic reproductive physiology of this species.

Other characteristics of captive management of prosimians and New World species, including nutritional needs, will not be discussed in this chapter as they are covered in other chapters of this volume. Similarly, emphasis on reducing the stress of captivity, an obviously desirable characteristic, has not been extensively discussed; readers are referred to Bernstein (1989) for a description of the objective measurement and assessment of well-being in nonhuman primates.

B. Old World Species and the Great Apes

A wide variety of approaches have been undertaken to establish breeding colonies of Old World species. The literature is replete with detailed accounts concerned with behavior and breeding outcomes of various species under many different housing conditions. The intent of this section is to provide a broad overview for a limited number of species which are commonly used under semi-free-ranging and domestic conditions.

1. Group Mating

The rhesus monkey provides ample information on free-ranging, outdoor field cages (corrals), and indoor breeding colonies because of its extensive use during the past half century. Free-ranging colonies of *M. mulatta* have been maintained on the island of Cayo Santiago, Puerto Rico, since the 1940s (Carpenter, 1972; Rawlins and Kessler, 1986). Much of what is known about rhesus monkey social and reproductive behavior comes from studies of this colony. Moreover, it is one of the very few facilities that was dedicated as a unique site for the study of primate biology under seminatural conditions. Studies between 1959 and 1964 indicated that the annual population increase was 16% with an annual mortality rate of 6.5%. The mean live birth rate for mature females was 78.5% and the mean annual infant mortality rate was 8.5%. Animals were routinely removed from the colony and intermittent trapping occurred; it was felt that these events had little effect on the colony (Koford, 1965). The population on Cayo Santiago was left intact from 1972 to 1984 and all animals were of known identity, age, sex,

TABLE I

REPRODUCTIVE CHARACTERISTICS OF 58 PRIMATE SPECIES[a]

Common name	Scientific name	Adult body weight (g)	Neonate weight (g)	Young per litter	Interbirth interval (years)	Female age at first reproduction (years)	Maximum life span (years)
Prosimians							
Galago							
Demidoff galago	*Galago demidovii*	64.7	7.5	1.2	1.0	1.0	9.0
Greater galago	*Galago garnetti*	779.6	49.7	1.0	0.6	1.3	17.0
Lesser galago	*Galago senegalensis*	190.5	11.5	1.6	0.6	0.9	16.5
Lemur							
Fulvus lemur	*Lemur fulvus*	2,200.5	74.4	1.0	1.5	2.3	30.1
Mouse lemur	*Microcebus murinus*	66.5	6.5	1.9	0.9	1.0	15.4
Ring-tailed lemur	*Lemur catta*	2,196.5	85.8	1.2	1.4	2.0	27.1
Ruffed lemur	*Varecia variegatus*	3,100.0	97.2	1.8	1.0	1.7	13.0
Potto	*Perodicticus potto*	953.5	46.5	1.1	1.0	2.0	22.3
Sifaka	*Propithecus verreauxi*	3,384.0	107.0	1.0	1.0	2.8	18.2
Slender loris	*Loris tardigradus*	226.0	11.4	1.6	0.5	1.5	12.0
New World monkeys							
Cebus							
Black-capped cebus	*Cebus apella*	2,741.0	239.7	1.0	1.8	5.5	44.0
White-fronted cebus	*Cebus albifrons*	2,490.5	234.0	1.0	1.5	4.0	44.0
Golden lion tamarin	*Leontopithecus rosalia*	559.6	50.0	2.0	0.5	2.4	14.2
Mantled howler	*Alouatta palliata*	6,583.5	480.0	1.1	1.9	3.6	20.0
Marmoset							
Common marmoset	*Callithrix jacchus*	288.0	27.0	2.1	0.5	1.5	11.7
Cotton-top marmoset	*Saguinus oedipus*	416.5	44.0	1.9	0.6	1.9	13.5
Goeldis marmoset	*Callimico goeldii*	582.0	50.6	1.0	0.5	1.3	9.3
Pygmy marmoset	*Cebuella pygmaea*	71.5	15.0	2.1	0.5	1.9	11.7
Owl monkey	*Aotus trivirgatus*	733.5	97.0	1.0	0.7	2.4	20.0
Spider monkey	*Ateles geoffroyi*	7,576.0	426.0	1.0	0.5	5.0	27.3
Squirrel monkey	*Saimiri sciureus*	752.0	95.2	1.0	1.1	2.5	21.0
Woolley monkey	*Logothrix lagothrix*	6,127.5	450.0	1.0	1.5	5.0	25.9
Old World monkeys							
Blue monkey	*Cercopithecus mitis*	5,827.0	402.5	1.0	1.3	4.3	20.0
Celebes black ape	*Cynopithecus niger*	5,400.0	455.0	1.0	1.5	4.8	18.0
De Brazza's monkey	*Cercopithecus neglectus*	5,558.0	260.0	1.0	1.6	4.0	22.0
Gelada baboon	*Theropithecus gelada*	15,069.0	553.0	1.0	2.1	4.0	19.3
Green monkey	*Cercopithecus aethiops*	4,173.5	314.0	1.0	0.9	3.5	24.0
Macaques							
Cynomolgus macaque	*Macaca fascicularis*	4,532.0	345.0	1.0	1.1	3.9	37.1
Japanese macaque	*Macaca fuscata*	10,450.0	496.0	1.0	1.5	5.5	33.0
Pig-tailed macaque	*Macaca nemestrina*	7,761.0	472.0	1.0	1.1	3.2	26.3
Rhesus macaque	*Macaca mulatta*	5,906.5	475.0	1.0	1.0	5.0	29.0
Stump-tailed macaque	*Macaca arctoides*	9,286.5	487.0	1.0	1.5	3.5	30.0
Mandrill	*Mandrillus sphinx*	16,440.0	613.0	1.0	1.4	5.0	28.6
Mangabey	*Cercocebus albigena*	7,362.0	425.0	1.0	2.1	4.1	32.7
Patas monkey	*Erythrocebus patas*	9,458.5	504.5	1.0	1.2	2.6	21.6
Talapoin	*Miopithecus talapoin*	1,250.0	175.5	1.0	1.0	4.4	27.7
Great apes							
Chimpanzee	*Pan troglodytes*	45,900.0	1,742.0	1.0	3.1	11.2	53.0
Gibbon	*Hylobates lar*	5,555.5	400.0	1.0	2.7	9.3	31.5
Gorilla	*Gorilla gorilla*	117,549.4	2,122.9	1.0	4.1	7.2	50.0
Orangutan	*Pongo pygmaeus*	55,233.0	1,735.0	1.0	1.0	10.7	57.3
Siamang	*Symphalangus syndactylus*	10,827.0	517.0	1.0	3.0	9.0	35.0
Humans							
Man	*Homo sapiens*	60,000.0	3,375.0	1.0	3.5	14.0	100.0

[a]From Ross (1988).

and maternal geneology. In addition, life histories on each animal were kept via a daily census, and data on births, morbidity, mortality, and group affiliation have been recorded. The population increased from 479 to 1161 animals for a net gain of 142.3% from July 1, 1976, to June 30, 1983 (Rawlins and Kessler, 1986). The per annum net increase was 13%. The sex ratio of the entire colony was reported as 103 males to 100 females and the mean annual mortality rate was 6.8%. A total of 1171 births have been recorded during the 8 birth seasons. The average interbirth interval for females is 372 days for those who delivered and weaned their offspring in consecutive years compared to 336 days for those females who suffered stillbirths or abortions or whose infants died prior to weaning. Of particular interest is the annual net reproductive rate, which ranged between 74.1 and 84.7%, with a cumulative mean of 80.3%. Of the total births, 95.7% were live births, 0.7% of the live births died within 2 days of life, and 4.3% were aborted or stillborn. The mean annual survivorship was 92.5 and 92.8% for male and female infants, respectively, and the mean annual survivorship for both sexes to 1 year of age was 92.7%. These data suggest that semi-free-ranging *M. mulatta* are more successful in producing liveborn infants than those kept in smaller enclosures (see the following discussion).

A semi-free-ranging colony of *M. mulatta* has been maintained for a decade and a half on Key Lois, Florida, an island in the Florida Keys. Several large corrals are used for holding and trapping animals. An excess of 1800 animals were organized into four troops in 1978 (Pucak, 1978). The colony was composed of 1350 adult breeders and over 500 young animals less than 2 years of age. Fetal and infant deaths were reported to be minimal, e.g., in 1976 17 of 344 (5%) newborn infants died before 6 months of age.

Another very successful free-ranging population of *M. mulatta* is located on a 400-acre densely wooded island off the coast of South Carolina (Taub and Mehlman, 1989). The Morgan Island colony began in 1979 when 1400 animals were translocated from the La Parguera facility of the Caribbean Primate Research Center. Over a 9-year period, the colony grew almost fourfold to roughly 4000 animals; during this time over 6000 infants were born on the island and approximately 3150 were shipped to other government biomedical programs. The 9-year average pregnancy and live birth rates for the entire colony were 78 and 71%, respectively. Corresponding rates of fetal and neonatal losses were 8 and 5%, respectively. Juvenile females also reproduced well, averaging 76% pregnancy and 70% live birth rates.

The California Primate Research Center (CPRC) has had an active breeding program of rhesus monkeys in outdoor cages since 1972 (Hendrickx and Henrickson, 1988; Small and Smith, 1986) and, with the ban on exportation of rhesus monkeys by India in 1978, the breeding program was expanded to a total of 10 field cages housing approximately 800 animals. The field cages are one-half acre enclosures (200 × 100 × 8 feet or 67 × 33 × 2.6 meters) constructed of chain-link fencing material and include a holding cage and chute for separating animals. Elevated A-frame wooden shelters are located throughout the cage and the corners are reinforced with sheet metal for additional protection from wind and rain. Horizontal perches are provided for cage enrichment.

The total number of animals per field cage varies from cage to cage and also varies from season to season due to deaths, births, and harvest schedules. The average number of adult males in six cages selected for analysis from 1984 to 1988 was 12 per field cage (Hendrickx and Henrickson, 1988). The average number of females for the same time period was 35 per field cage and the total number of animals varied between 650 and 680. Although these groups vary in number for each age category, they are considered seminatural in their composition, social structure, and dynamics. The number of infant males is usually reduced to two to four per cage at the end of the breeding season by relocation to other areas. All surviving female infants are maintained in the field cages and a very small number of both sexes are cross-fostered to other field cages to minimize inbreeding.

Six field cages selected for study were formed between 1976 and 1982, and included one cage of wild-caught animals from India, one cage containing a mixture of wild-caught animals from India and animals bred and reared at the CPRC, and the remaining four cages were composed of animals bred and reared at the CPRC or bought from the Yamasee Primate Research Center, Yamasee, South Carolina, where they were bred and reared on an island in a seminatural environment. At present, most of the animals are first to fifth generation offspring of the original groups.

Conception was recognized if one of the following outcomes was recorded: live birth, stillbirth (>140 days gestation), and abortion (<139 days gestation). Reproductive rates were calculated as:

$$\text{Conception rate} = \frac{\text{number of pregnancies in adult females}}{\text{average number of adult females at risk of pregnancy}} \times 100,$$

$$\text{Live birth rate} = \frac{\text{number of live births}}{\text{total number of conceptions (pregnancies) in same period}} \times 100,$$

$$\text{Stillbirth rate} = \frac{\text{total number of stillbirths for specified breeding season}}{\text{total number of conceptions (pregnancies) in same period}} \times 100,$$

$$\text{Abortion rate} = \frac{\text{total number of abortions in specified breeding season}}{\text{total number of conceptions (pregnancies) in same period}} \times 100.$$

TABLE II

REPRODUCTIVE OUTCOME IN ADULT FEMALES FOR FIVE BREEDING SEASONS (1984–1988)

Cage	Reproductive rates (mean % ± SD)			
	Conception	Live birth	Stillbirth	Abortion
2	84.5 ± 9.9	87.8 ± 6.2	7.8 ± 3.5	2.7 ± 2.7
3	92.6 ± 4.5[a]	86.0 ± 6.8	10.2 ± 5.2	2.8 ± 3.3
4	85.1 ± 10.5	84.7 ± 13.9	6.6 ± 4.8	7.4 ± 9.4
5	85.8 ± 6.2	85.1 ± 6.2	7.2 ± 3.5	6.3 ± 3.2
7	84.1 ± 10.1	85.3 ± 4.5	6.7 ± 4.4	6.2 ± 4.9
9	79.8 ± 10.9[a]	81.0 ± 12.1	8.1 ± 7.4	9.5 ± 7.1

[a] $p<0.05$.

The average population of each field cage was calculated by taking an average of the monthly population census over the entire time period under evaluation. The variations in populations were dependent on a number of factors, including the original number of animals comprising the field cage, the social dynamics, live birth rate, infant sex, morbidity, mortality, and harvesting for research needs. All of these factors, especially changes in social interactions (i.e., fighting) of the groups within each field cage, influence stability and ultimately the pregnancy outcome. All six field cages were considered as having similar populations with respect to reproductive potential.

Conception rates varied considerably from year to year and from cage to cage. All six field cages achieved the anticipated minimum conception rate of 70% over the 5-year study period. When conceptions for all six field cages over the 5-year study period were compared (Table II), only field cage 9 was significantly different ($p<0.05$) from field cage 3 but was not different from the other field cages. Although no differences in abortion rate were found among the six field cages during the entire 5-year study period, considerable variation was noted between and within individual field cages. The abortion rate is the least reliable factor in determining pregnancy outcome for several reasons. Palpation for pregnancy, the only procedure used in the field cages, was performed only once during the breeding season (spring roundup); thus, pregnancy is not confirmed in some animals and early abortions may also be undetected by this method. The live birth rate was quite consistent, varying <7% among cages over the 5-year period. Similarly, significant differences were not observed in the stillbirth rates (Table II). The same yearly range of variation was noted within each field cage as with the other parameters studied. Births occurred over a 4.5- to 5.5-month period with the peak of the birth season usually occurring from early March to late May. Data indicate that the birth season probably does not influence conception or pregnancy outcomes in subsequent seasons or, conversely, that these parameters do not influence the birth seasons with regard to onset, pattern, or duration of births.

These data show that there are no essential differences in the three parameters measured (abortion, stillbirth, and live birth rates) among the six cages, regardless of the origin of the animals or type of housing during the first year of life. The only difference observed was in the conception rate between field cage 3 originally formed of animals composed of adults bred and reared at the CPRC and field cage 9 populated with nulliparous females, many of whom were reared in the CPRC nursery. These data suggest that infants reared with their mothers and then placed in social groups initially have a higher conception rate, at least in their early years, than that of infants separated at birth from their mothers. Even though the later group was reared in similar social situations after 6 months of age, the absence of the mother–infant interaction at a very early age may delay the maturational process which in turn is demonstrated in a reduced conception rate early in adulthood.

The large-scale breeding of baboons (*Papio* spp.) has been successfully undertaken for many years at the Southwest Foundation for Research and Education, San Antonio, Texas. Using a unique 6-acre corral facility, approximately 200 infants per year have been produced (Goodwin and Coelho, 1982). Total live birth production was 81% over a 2-year period. Births occurred in all months of the year with the greatest number occurring between June and December. Both stillbirths (~25%) and postnatal mortality (>25%) were highest in January, February, and March, the coldest months in South Texas. The general management of the field cages/corrals was similar at both Davis, California, and San Antonio, Texas, in that the policy was to provide care and to intervene only when necessary. Observations were made daily by animal technicians to identify sick or injured animals as well as to record births and social disruptions. Ill or wounded animals were captured for treatment. All animals were captured and evaluated three times per year for management and research information. The Washington Primate Research Center maintains a breeding colony of *P. cynocephalus* at the primate field station in Medical Lake, Washington. Animals are housed in corrals containing a heated shelter which has proven effective in a location with severe winter weather (Goodwin, 1986).

Efforts in the Philippines, known as the Siconbrec Project (Simian Conservation Breeding and Research Centre), have indicated that *M. fascicularis* are being bred in corrals of varying sizes on a large scale in the country of origin (Hobbs *et al.*, 1987; Hobbs, 1989). A 94% live birth rate was realized over a 34-month period (1985–1987) involving more than 1600 females, and 77% of the live births survived until weaning. A similar breeding operation is being conducted in the United States at Yamasee, South Carolina. A number of other facilities (Oregon, Delta, and Yerkes Regional Primate Research Centers) have used field cages or corrals of varying sizes successfully for the production of various macaque species (*M. fuscata, M. mulatta, M. nemestrina, M. radiata*) (Goodwin, 1986). At the University of Texas System Cancer Center in Bastrop, Texas, a large outdoor corral (built to accommodate 130 animals)

attached to indoor housing is used for harem breeding and for rehabilitation of chimpanzees (Martin, 1981).

Group of harem mating systems have been used extensively for the production of *M. fascicularis, M. mulatta, M. nemestrina, C. aethiops*, and *Papio* spp. Macaque species, especially *M. mulatta* and *M. fascicularis*, have been housed in structures built to store corn; these "corn cribs" have been modified over time to provide more protection from inclement weather and enrichment for the animal occupants. At the CPRC in Davis, California, two circular corn cribs 16 feet (5.5m) in diameter and 16 feet high are connected with intercage units which provide direct communication through a chute that is permanently located at the bottom of the rear wall of the intercage unit. The chute can be opened or closed as needed for housing groups of various sizes or for behavioral or medical reasons. Twelve to 24 animals can be accommodated in this configuration, depending on group composition (Hendrickx and Henrickson, 1988). For *M. mulatta* the conception, abortion, stillbirth, and live birth rates are similar to those observed in the field cages; the live birth rate for 1988–1990 was 85%.

A harem mating system, consisting of two groups of 10 females and 1 male each, was established to breed cynomolgus monkeys in Malaysia (Werner *et al.*, 1980). The breeding building containing two rooms was constructed of wood support posts with wire mesh walls, zinc sheet roofing, and a concrete slab floor. Over a 3-year period there were 39 live births, 1 stillbirth, and 11 neonatal deaths. The average time to wean the 28 remaining offspring was 230 days and the average time to conception postweaning was 50 days. Of the three weaning systems evaluated, the best method was caging the mother–infant pair within or adjacent to the breeding room followed by a two-part cage system which allowed the infant to continue nursing and also obtain solid food inaccessible to the mother. Harem breeding in combined outdoor/indoor and indoor caging facilities has also been successfully used to produce rhesus and long-tailed macaques (Balner, 1975; Valerio and Dalgard, 1975). *M. fascicularis* obtained from Malaysia and Indonesia were mainly housed in indoor–outdoor enclosures as social groups of 1 adult male and 7 to 10 females for research on atherosclerosis (Gardin *et al.*, 1989). The overall breeding performance for this colony, which is located at the Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, North Carolina, was a pregnancy rate (number of pregnancies divided by total number of female years) of 53%, a stillbirth rate of 22%, and an infant mortality rate of 22%; 60% of the infants that were conceived lived more than 12 months. Although maternal age did not affect reproductive outcome, the pregnancy rate peaked between 6 and 8 years of age, and the lowest infant mortality and stillbirth rates were associated with 9- to 11-year-old females. Neither stillbirth rate nor infant survival was associated with maternal origin.

Harem mating of *M. nemestrina* has been practiced at the Regional Primate Research Center Field Station, Medical Lake, Washington, since the 1970s. A harem, generally composed of one adult male and six to eight females, was housed indoors in rooms measuring 2.2 × 3.4 m and employing a light:dark cycle of 14:10 hr. New groups were established by introducing all animals into the room simultaneously (Blakley *et al.*, 1972). Over a 12-year period the live birth rate among 412 females and 94 male breeders was 72.7%; there was a 14.4% incidence of abortion and stillbirth and a 12.9% neonatal death rate (Sackett *et al.*, 1975). Production of known gestational-age fetuses was obtained by pairing females with proven sires during midcycle. Almost 100 pregnancies were produced in a 3-year period by this method.

Successful breeding colonies of African green monkeys (*Cercopithecus aethiops aethiops* and *C. aethiops pygerythrus*) have been established in the United States (Pennsylvania) and in Kenya (near Nairobi) (Else, 1985; Kushner *et al.*, 1982), although the success rate is varied. The colony maintained near Philadelphia, Pennsylvania, was initially composed of feral animals housed in small enclosures (4 × 5 × 7 feet) connected to a larger exercise area (5 × 18 × 7 feet). Each breeding group contained 1 male and 2 to 6 females; pregnant females were removed from their breeding groups prior to the expected date of delivery and the mother–infant pairs were returned to their breeding group within 2 weeks of birth. Collectively, of the 221 pregnancies recorded for the two subspecies, 186 (84%) delivered viable infants and 35 (16%) had stillbirths and abortions; 23 (12%) of the newborns (live births) died within 150 days postpartum. Harem groups composed of 1 male and 5 to 10 females housed in outdoor enclosures measuring 3 × 6 × 2.5 m had an average conception rate of 87.6% (64 of 73) over a 3-year period (1981–1983) (Else, 1985). The live birth rate was 85% (two stillbirths out of 64 total births). Thirteen (21%) infant deaths occurred within a month of birth of which five were attributed to lactation failure in the mothers. The annual production rate (number of births per year surviving to 6 months of age divided by the total number of females) was 67%. The authors emphasized the importance of conditioning for this species, which is prone to stress, for at least 1 year before regular cyclicity and subsequent conception occur.

The brief summary just provided for several Old World species indicates that a high level of production under a variety of conditions can be achieved with the proper husbandry and management.

Twenty to 25 females and 1 to 2 male baboons have been used to make up a harem group in outdoor caging facilities (Moore, 1975). Several days were required for a new breeding group to establish a social structure. Altercations were frequent but usually only minor wounds were inflicted. Once the breeding groups was established, the live birth rate was usually above 70%. In most instances, the pregnant animals were removed 3 weeks before anticipated parturition, which was based on deturgescence of the sex skin, as described next, and placed in individual cages for delivery.

A novel cage design has been developed by Else *et al.* (1986) in order to provide permanent breeding groups continuous so-

cial contact, and also to confine females for urine collections in individual cages. Under these conditions, 60 out of 67 females (90%) became pregnant and 51 conceived again following hysterotomy between Gestational Days (GD) 35 and 100. A total of 181 pregnancies were recorded and 15 (14%) ended in abortion.

Harem breeding of chimpanzees was established at the Southwest Foundation for Research and Education, San Antonio, Texas, in 1967 and infant production started in 1970. The animals are housed in indoor–outdoor facilities accommodating one male to three or four females. Between 1970 and 1980, 132 total births occurred; 113 (86%) of these were live births and 16 (12%) were stillbirths (Martin, 1981). Immunogenetic studies dictate the breeding strategies used at the Dutch Primate Center where harem-style breeding of chimpanzees is also done in indoor–outdoor cages. Sixty-one births were recorded between 1971 and 1978 which included 56 live births (92%) and 5 stillbirths (8%) (Martin, 1981).

2. Timed Mating

One of the primary objectives of most nonhuman primate breeding colonies is to produce timed pregnancies in which the age of the conceptus is known. Pregnancies of known age are very important for research in reproductive biology, endocrinology, teratology, and developmental toxicology. Accurate aging (staging) of the pregnancy is accomplished by employing a number of different methods. Among the more common methods are restricting the time the female and male are housed together during the ovulation period, the so-called "timed" or "calendar" method of mating (Hendrickx and Kraemer, 1970). Some of the more commonly used systems in different facilities are described next; it is important for the reader to bear in mind that the system used is often adopted to meet specific program needs but usually addresses two important points: (1) to provide an accurate means of dating the pregnancy, and (2) to increase the conception rate and thus decrease the cost of maintaining the breeding colony by providing more pregnancies over a defined period of time (i.e., 1 year).

Van Wagenen (1972) was one of the first to time mate macaques. The female was placed in the male's cage on the 11th day of the menstrual cycle and remained there until the 12th day when a vaginal lavage was taken and examined for sperm. If sperm were present, the mating was called positive. If pregnancy was established, Day 1 of gestation was designated as the day sperm were found in the vaginal lavage. Almost 700 pregnancies, including normal as well as abortuses and experimental pregnancies, were produced by this method. Most of the timed-mating procedures presently employed are a variation on the approach taken by van Wagenen (1972).

The CPRC employs several timed-mating strategies to meet its research needs and to maximize the conception rate. The most common mating schedule for both *M. mulatta* and *M. fascicularis* is to place the female in the male's cage every other day (two times) over a 3-day period for 2 hr. The calculated optimal day for conception is scheduled as the day between the two matings; the optimal mating days are either menstrual cycle Days 10 and 12 or 11 and 13 as determined by the length of the previous cycles. The formula for breeding is obtained by dividing the average menstrual cycle length of the last three cycles by two and subtracting three to give the optimum cycle day of mating. Day 1 is the onset of menses. Variations of this formula may be used to accommodate specific animals. One such variation is to place the female in the male's cage every other day for a 5-day period (three times) for 2 hr. The calculated optimum day for conception is scheduled as the middle day of the breeding period which is considered the conception date (Day 0), unless results of hormone assays (see the following discussion) indicate that the conception date should be changed. Using this method, the conception rate (number of pregnancies divided by number of adult females) for *M. mulatta* (N=541) exceeded 70%, and for *M. fascicularis* (N=216) was greater than 60% despite the fact that large numbers of females were not mated for 2 to 3 months due to scheduling delays related to large developmental studies.

The main purpose of limiting the pairing of the male and female is to conserve the male for mating with other females. Both macaque species, but especially *M. fascicularis*, tend to copulate within minutes of being paired. In most programs, breeding females and males are housed in the same room since visual and olfactory cues are important in male–female sexual attractiveness, in addition to decreasing the spread of disease between animal rooms. However, several facilities (i.e., Oregon Regional Primate Research Center, Beaverton, Oregon) have had very successful *M. mulatta* breeding programs by keeping all the males in one room (a stud room) and bringing the females there for mating.

Single matings (one-time mating) are scheduled for the optimal day for conception as determined by the just-referenced formula. With regard to single matings, little or no difference was observed in developmental stages and embryonic age of rhesus monkey embryos obtained by cesarean section between GD 25 and 46 following male–female pairing for 2, 4, or 24 hr (Hendrickx *et al.*, 1975). In fact, the correlation of developmental stage and embryonic age for rhesus monkey embryos obtained by cesarean section between GD 22 and 49 was similar when single matings and multiple matings (three matings, every other day for a 5-day period) were compared (Fig. 1).

Honjo *et al.* (1984) have provided extensive data regarding reproductive parameters for *M. fascicularis* housed individually indoors at the Tsukuba Primate Center for Medical Science, Tsukuba, Japan. The total gestation rate (number of pregnancies divided by frequency of mating) was 55.1%; the gestation rate for feral females was 57.9% (N=1309) compared to 36.8% (N=128) for F_1 females. The reduced gestation rate for F_1 females was partially attributed to poor mating behavior. The total abortion rate for both feral and F_1 animals was 4.7%; the abortion rate for feral animals was only 4.2% (N=50) compared to

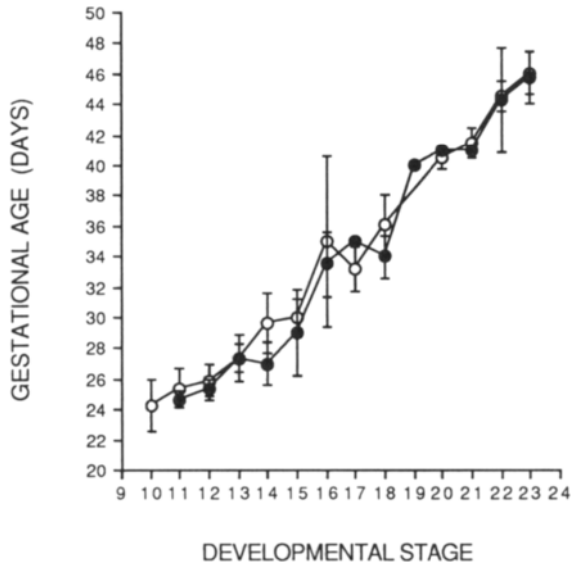

Fig. 1. Correlation of developmental stage and gestational age (mean days ± standard deviation) in embryos of rhesus monkeys that were single-mated (○; $N = 68$) versus multiple-mated (●; $N = 31$). There was no marked difference in this relationship between the two methods of breeding.

9.9% ($N=12$) for F_1 females. Similarly, feral females yielded a stillbirth rate of 8.6% compared to 12.5% for the F_1 animals; the total stillbirth rate was 8.9%. A relatively high weaning rate of 97.2% ($N=939$) and 89.2% ($N=83$) for feral and F_1 females was experienced. Weaning from nursing mothers was scheduled at least 3 months after birth; infant mortality was about 3.4% prior to the time of weaning.

In baboons, the average conception rate (number of conceptions divided by number of matings) has been reported to be 35% (Moore, 1975), although matings on the third day preceding deturgescence (Day 3 deturgescence) have yielded a conception rate of 48% (Hendrickx and Kraemer, 1969). The annual conception rate (see preceding discussion) usually exceeds 70% for baboons and macaques.

Timed matings of chimpanzees have been accomplished at three facilities in the United States (Martin, 1981). The oldest of these is at the Yerkes Regional Primate Research Center, Atlanta, Georgia, where most of the chimpanzees are housed as pairs in an indoor–outdoor cage arrangement separated by a guillotine door. A small colony ($N=15$) has also been housed in a semi-free-ranging condition at a nearby experimental field station. One hundred and forty three total chimpanzee births have been recorded from 1965 to 1979; an additional 161 births occurred in the laboratory directed by Robert M. Yerkes during the 35 years it was located at Orange Park, Florida, before it was moved to Atlanta. The abortion and stillbirth rates were about 10% during this time. Four generations have been produced and an approximate generation time of 13 years has been calculated. The incidence of multiple births is about 1 in 24, a much higher incidence of twinning than in human births in the United States.

An active breeding program began at the Primate Research Institute, New Mexico State University, Holoman AFB, New Mexico, in 1966 and is now recognized as the largest chimpanzee breeding program in the world. Matings between male/female pairs are accomplished in a manner very similar to that reported at the Yerkes Center. More than 160 live births were reported between 1966 and 1978 and the stillbirth rate was approximately 15% (Martin, 1981).

At the Laboratory for Experimental Medicine and Surgery in Primates, Tuxedo, New York, chimpanzees are maintained individually in hanging cages. Menstrual cyclicity and perineal sex skin characteristics are both recorded, and paired matings are planned according to blood groups and social compatibility. Short-term matings are scheduled when accurately timed pregnancies are required. A live birth rate of 83.3% (45 of 54), a stillbirth rate of 13% (7 of 54), and an abortion rate of 3.7% (2 of 54) have been reported; no second generation offspring have yet been realized (Martin, 1981).

III. DETECTION OF ESTRUS AND MENSES

A. Prosimians and New World Species

Traditionally, estrus has been evaluated by changes in vaginal cytology, behavioral characteristics of the female, or steroid levels in blood or urine. The phases of the estrous cycle have been well-described in the greater bushbaby (Hendrickx and Newman, 1978). Although the vaginal orifice is sealed for a substantial part of the estrous cycle in this species, specific cyclic changes occur in the external genitalia, including changes in color and swelling of the labial folds and vulvar regions. These characteristics, correlated with changes of the cells in the vaginal lavage, were used to divide the estrous cycle into four phases (proestrus, estrus, metestrus, and diestrus). Sexual receptivity was usually limited to the first several days of the estrous cycle.

Black lemurs (*Lemur macaco*) and ruffed lemurs (*Varecia variegata*) have been monitored for cyclic changes in behavior, size of external genitalia, and vaginal cytology obtained by lavage. Detection of estrus in ruffed lemurs is relatively easy since the vulva remains small and completely imperforate except just prior to, during, and immediately after estrus (Bogart *et al.*, 1977). Vaginal smears have also been obtained in the slender loris (*Loris tardigradus*) to ascertain the cycle phase (Izard and Rasmussen, 1985). In some species, such as the potto, it is virtually impossible to detect estrus (Blackwell and Menzies, 1968). Since collection of routine serum samples from small species is difficult, utilization of urine samples for hormonal assessment in prosimians has proven valuable (Lasley *et al.*, 1980).

Reports on the use of vaginal cytology to assess estrus in the New World species have been variable, although some investigators have utilized the technique very effectively (Travis and Holmes, 1974). Behavioral estrus is difficult to measure in these highly stressful primates and very careful observation is required, probably far more than normally practiced with a breeding colony. Reliable morphological and behavioral indices of female cyclicity are not available for tamarins, marmosets, or squirrel monkeys; however, changes in vaginal cornification have been used to monitor the ovarian cycle in squirrel monkeys (Dukelow, 1983; Stolzenberg *et al.*, 1979). Attempts have been made to correlate cyclic swelling of the external genitalia or sexual receptivity with estrogenic activity as determined by vaginal smears, but a clear-cut relationship between these observations and ovulation has not been made (Castellanos and McCombs, 1968; Wolf *et al.*, 1975). The capuchin (*Cebus apella*) has a menstrual cycle, although uterine bleeding, which is not always overt, must often be verified microscopically (Wright and Bush, 1977). Characteristic changes in the number of neutrophils and erythrocytes as well as the appearance of epithelial cells in vaginal smears are used in determining the phases of the menstrual cycle in this species (Nagle and Denari, 1983). These cytological changes were correlated with the plasma profile of 17β-estradiol and progesterone. The evaluation of urine and feces for excretion of estrone, 17β-estradiol, and progesterone has been utilized for estrus evaluation in the squirrel monkey (Travis and Holmes, 1974), marmoset (Hearn, 1983), tamarin (Heistermann *et al.*, 1987; Ziegler *et al.*, 1987, 1989), and owl monkey (Dixson, 1983).

B. Old World Species and the Great Apes

In contrast to prosimians and New World species, ovarian cyclicity is easily determined in the higher primates due to an overt menses. In almost all breeding colonies, monitoring menses is done in one of two ways: (1) visual examination of the external genitalia for fresh blood, or (2) use of vaginal swabs to detect menstrual blood. Vaginal swabs or smears are taken by placing a cotton-tipped swab into the vaginal canal, preferably in the anterior fornix, and then visually examining the swab for the presence of blood. In some instances it may be beneficial, especially in young pubertal or oligomenorrheic animals, to smear the swab on a glass microscope slide and examine it for red blood cells.

In approximately 16% of the menstrual cycles of *P. cynocephalus*, overt menstruation does not occur in successive cycles, making it difficult to utilize gross observation as an end point without collecting vaginal smears (Hendrickx, 1971). Although vaginal smears make it possible to detect menstruation in approximately 95% of the cycles, it is less convenient to measure than the changes that occur in the perineum (sex skin). The visible cyclic changes in the sex skin or perineum which correlate with the menstrual cycle have been well-described in the baboon (Hendrickx, 1971). The turgescent phase encompasses an initial turgescent stage (average 4 days) when the perineal area starts to swell with a decrease in wrinkling of the skin which changes color from dull pink to a pinkish red. During the subsequent maximum turgescent stage (average 13 days) the skin of the perineum is fully distended with no wrinkles and attains its deepest and most intense bright red color (Fig. 2a). The deturgescent phase is similarly divided into two stages. Initial deturgescence (average 5 days) begins with a loss of color, a decrease in size of the swelling, and a corresponding increase in wrinkles (Fig. 2b). During the following quiescent stage (average 12 days), the perineum is of minimal size and the labia and clitoris have many wrinkles with an overall pinkish-red color. The dull epithelial surface of the perineum, which begins to slough during the deturgescent stage, is usually completely shed by the end of the quiescent phase.

The sex skin (perineal swelling) is also a very reliable way of accurately dating the pregnancy in several Old World monkeys (baboon, pig-tailed macaques, and others) and the chimpanzee. Timed matings in *P. cynocephalus* have established that Day −3 deturgescence (the third day before the onset of deturgescence) is the optimal day for mating (Hendrickx, 1971). Endocrinological data indicate that ovulation occurs most often on Day −1 or −2 deturgescence (Shaikh *et al.*, 1982; Wildt *et al.*, 1977), therefore Day −1 or −2 should be designated as Day 0 of pregnancy. Bielert *et al.* (1976) have demonstrated a positive correlation among sex skin color, circulating levels of estradiol, and increased sexual activity as indicated by ejaculations in periovulatory rhesus monkeys.

In female chimpanzees the sex skin (genital swelling) can be monitored using a scale of 0 (minimum) to 3, 4, or 5 (maximum), depending on the laboratory, in a manner very similar to that described for the baboon (Martin, 1981). Monthly menstrual cycle charts are maintained for each animal and various male–female pairings are made for several months at a time; if pregnancy does not occur, different pairings are attempted. Day 0 of pregnancy is equivalent to the last day of maximal turgescence using this method.

IV. DETECTION OF OVULATION

As discussed in Chapter 9, ovulation occurs with few, if any, behavioral or externally perceptible cues, yet the ability to predict ovulation is essential to many reproductive and developmental studies. Ovulation is normally detected by observation with a laparoscope, via ultrasonography, or by endocrinological measures.

While the science of laparoscopy is quite old, it has only been since the mid-1970s that it has been extensively used to view the internal organs. Among the prosimian species, greater galagos have been evaluated laparoscopically in addition to a number of the New and Old World species (Dukelow, 1975;

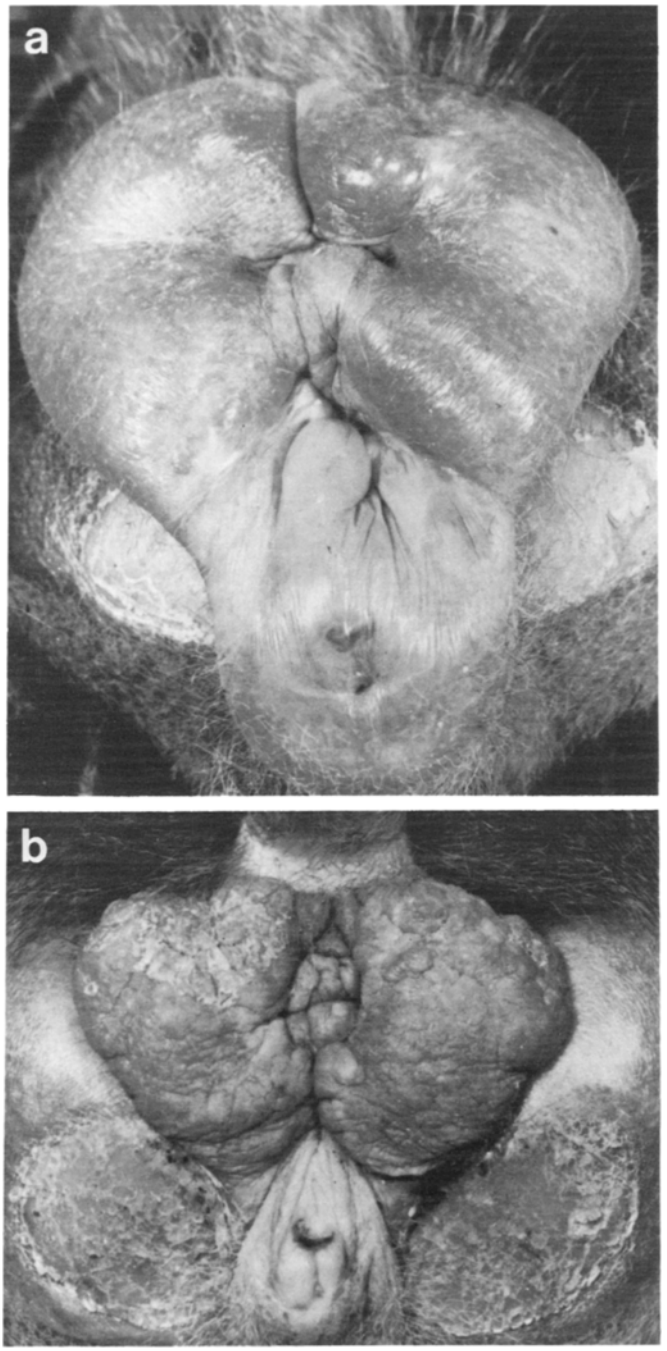

Fig. 2. Perineum of an adult baboon at two different stages of the menstrual cycle. (a) Maximum turgescent stage characterized by full distension of the perineal skin which has a smooth, shiny appearance and is a deep, intense red color. (b) Late turgescent stage identified by a loss of turgidity and color and an increase in perineal wrinkles.

Dukelow and Ariga, 1976; Dukelow *et al.*, 1973). This procedure involves anesthesia of the animal and insertion of a laparoscopic telescope into the abdominal cavity with subsequent insufflation with an inert gas, which allows visualization of abdominal structures including the ovary. By this technique, it is possible to evaluate developing follicles, sites of ovulation, or the presence of a corpus luteum. The dynamic changes in follicular structure and vascular patterns noted at laparoscopy have been used to diagnose the occurrence of ovulation within a short time interval (approximately 6 hr) in *Papio* spp. (Wildt *et al.*, 1977). Moreover, the sequential changes in ovarian follicular development have been correlated with perineal swelling, changes in vaginal cytology, and serum ovarian hormone levels (progesterone and estrogens). The use of these procedures is normally restricted in most breeding colonies to situations where the confirmation of ovulation is needed or determination of a pathological condition is required. It should be noted, however, that the potential detrimental effects of general anesthesia and carbon dioxide pneumoperitoneum on oocyte quality have been proposed (Lavy *et al.*, 1988).

Ultrasonography has been used as a means for imaging ovarian development in both the rhesus (Morgan *et al.*, 1987) and long-tailed macaque (VandeVoort and Tarantal, 1990). Because of the reliability for documenting the response to ovarian stimulation and aspiration of follicles, this procedure can be used for oocyte recovery for *in vitro* fertilization in these species.

A third method for detecting ovulation relates to the endocrinologic evaluation of urine or serum. During the normal estrous cycle, an estrogen peak occurs 15–24 hr prior to a peak in luteinizing hormone (LH). The latter triggers ovulation and the formation of the corpus luteum with a subsequent rise in progestins (see Chapter 9). The analysis of these hormones, i.e., the estrogens, LH, or progesterone, provides presumptive evidence that ovulation has occurred. Measures of urinary estrogen metabolites and LH have been reported for the capuchin monkey, squirrel monkey, marmoset, and tamarin (Harlow *et al.*, 1984; Heger and Neubert, 1988; Hodges *et al.*, 1979; Nagle *et al.*, 1980; Travis and Holmes, 1974; Ziegler *et al.*, 1987). Similarly, progesterone has been analyzed in the urine and serum of most of the more commonly used species such as the macaques (Monfort *et al.*, 1986, 1987). If ovulation does not occur, a number of techniques have been utilized to induce this process, namely treatment with exogenous gonadotropins (Dukelow, 1970, 1979; Kholkute and Nandekar, 1983; Kuehl and Dukelow, 1975) or gonadotropin-releasing hormone (GnRH) (Hodges *et al.*, 1988; Yeoman *et al.*, 1988). Prostaglandins have also been used to induce luteal regression (Hodges *et al.*, 1988). Most of these procedures are routinely used with domestic species and the common laboratory animals as well as humans. Thus, their application to nonhuman primates is appropriate.

A considerable body of evidence has been gathered regarding ovulation and its relationship to ovarian and pituitary endocrine events in *M. mulatta* (Hotchkiss *et al.*, 1971; Monfort *et al.*, 1987; Parkin and Hendrickx, 1975; Weick *et al.*, 1973), *M. radiata* (Lasley *et al.*, 1974; Parkin and Hendrickx, 1975), *M. fascicularis* (Behboodi *et al.*, 1990; Monfort *et al.*, 1987), *Papio* spp. (Shaikh *et al.*, 1982; Wildt *et al.*, 1977), and *P. troglodytes* (Gould and Faulkner, 1981).

Hotchkiss *et al.* (1971) reported that estradiol will rise over a 3-day period prior to ovulation in *M. mulatta*, indicating that

this parameter may be useful for predicting ovulation. Weick *et al.* (1973) have shown that plasma estradiol levels peak 9–15 hr prior to the preovulatory LH surge and approximately 30–40 hr before ovulation in the same species. Of particular relevance to the use of either ovarian hormone as a marker for ovulation are the observations by Bielert *et al.* (1976). These authors noted that sexual interaction increased between heterosexual pairs in daily time-limited matings which coincided with the preovulatory estradiol peak.

Additional information on the temporal relationship between the preovulatory estradiol peak and ovulation has been provided in *M. radiata* (Lasley *et al.*, 1974). Estradiol levels begin to rise 1 to 3 days prior to the peak and return to baseline within 2 days; additionally, estradiol peak occurred between cycle days 7 and 12 in 14 of 15 cycles. Ovulation was confirmed by the measurement of progestins which rose significantly the day following the estradiol peak. Observations at laparotomy confirmed that ovulation occurred 24–48 hr after the peak.

The favorable temporal relationship between the estradiol peak and ovulation, as well as the approximate 3-day duration of the preovulatory estradiol rise (Hotchkiss *et al.*, 1971), has made the measurement of this hormone a useful parameter for predicting ovulation. The introduction of rapid radioimmunoassays (RIAs) for total estrogens (E_{at}) in both serum and urine has further enhanced the practicality of relating optimal mating time directly to the preovulatory estrogen peak and thereby indirectly to ovulation (Parkin and Hendrickx, 1975). Although the precise temporal relationship between the E_{at} peak and breeding could not be determined, the optimal mating period for both *M. mulatta* and *M. fascicularis* appeared to be limited to an approximately 24-hr period at and immediately after the E_{at} peak.

RIAs for urinary estrone conjugates (E_1C) and progesterone metabolites have been developed and applied to the detection of ovulation and for monitoring reproductive function in *M. fascicularis* and *M. mulatta* (Monfort *et al.*, 1986, 1987). In *M. mulatta*, E_1C measurements in both nonconceptive and conceptive ovarian cycles demonstrate profiles that are both qualitatively and quantitatively similar to measurements of circulating serum estradiol. Thus, measurement of E_1C provides a practical and noninvasive approach in prospective and retrospective longitudinal studies of individual animals, providing adequate facilities for collection of urine are available. A 1.5 to 2.0-fold E_1C increase above the mean early follicular baseline has been observed 2 to 3 days before the E_1C peak which occurs in the majority of *M. fascicularis* between Days 8 and 15 of the menstrual cycle (Behboodi *et al.*, 1990). A single 2-hr mating before or the day of the E_1C peak resulted in a conception rate of 38.6%, which is comparable to a 40% conception rate in contemporary controls mated every other day over a 5-day period during midcycle (three times). In contrast, breeding 2 days prior to or 2 days after the peak significantly reduced the conception rate.

Hobson *et al.* (1976) have demonstrated the feasibility of mating rhesus monkeys after the preovulatory LH surge. LH was measured in daily serum samples by a rapid (24 hr) RIA using iodinated ovine LH as a tracer and an antiserum to human LH. When the assay results indicated a high LH value, the female was caged with a proven fertile male for 48 hr. The conception rate during the spring months was 43% compared to only 2.2% when females were bred according to the method of van Wagenen (1972) (i.e., females placed with males on menstrual cycle days 12 and 13).

Gould and Faulkner (1981) successfully modified the hemagglutination inhibition test for pregnancy detection to allow for detection of the midcycle LH peak in urine or serum samples of the gorilla, orangutan, and pygmy chimpanzee. Although a poor correlation between the LH peak and the last day of maximal swelling of the sex skin in the chimpanzee was not reliable for individual animals, data for the study group showed a maximal response for LH on the last day of maximal swelling and, thus, with the time of ovulation. By correlating the results of serial laparoscopy with the LH peak in this species, ovulation occurred 24 to 28 hr after the initial elevation of serum LH. This qualitative test provides a simple, rapid, and accurate means for detecting the midcycle LH peak in great apes (Gould and Faulkner, 1981).

Several temporal parameters, including body temperature, sex skin color changes, vaginal cytology, and cervical mucus, have been employed with varying degrees of success as indicators of ovulation. In general, the just-mentioned parameters lack the distinct, regular changes necessary to consistently signal an impending ovulation.

V. DETECTION AND MONITORING OF PREGNANCY

Before the early 1960s, the only means for diagnosing pregnancy in nonhuman primates was by palpation of the uterus. Hartman (1932) was the first investigator to describe in detail the method of bimanual rectal palpation for accurate determination of the stage of pregnancy by the size of the uterus, size of the fetal head, and to follow involution of the uterus postpartum. This procedure is still useful for diagnosing pregnancy in both indoor and outdoor breeding colonies as long as it is performed by well-trained and experienced individuals. The examination is best done under ketamine hydrochloride (Ketaset; 10 mg/kg) or similar anesthesia with the animal lying on its side or placed in the supine position (Fig. 3). In larger species (i.e., *M. mulatta, M. nemestrina, Papio* spp.) the examiner inserts the middle finger of the right hand into the rectum as far as possible, pressing toward the abdominal wall while the left hand is placed ventrally for counterpressure. In smaller species (i.e., *M. fascicularis*) use of the little finger may be more appropriate. The entire length of the uterus for most Old World monkeys, with the exception of large baboons, may be appreciated if nonpregnant or in the early stages of pregnancy; the ovaries may be identified on either side of the uterine body. The cervix

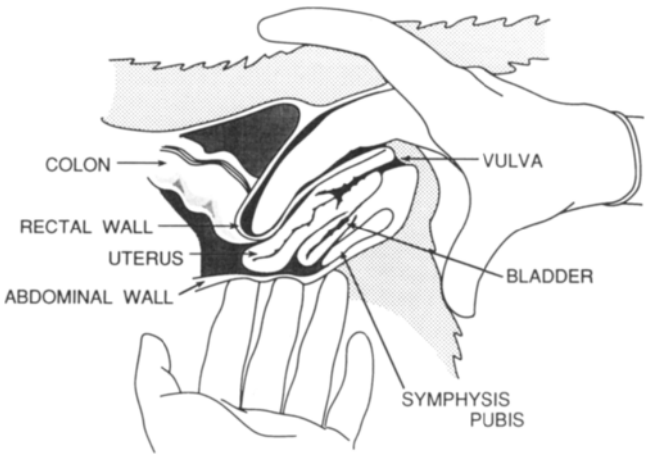

Fig. 3. Diagrammatic representation of bimanual palpation for pregnancy detection. Adapted from Wilson *et al.* (1970). From *Teratology*, 3. Copyright © 1970, John Wiley & Sons, Inc. Reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

in most macaques is readily distinguished by the sharp ridge marking its cranial border. The vagina is difficult to identify since it is collapsed against the symphysis pubis. If the ovaries are palpated, their size can be described by subjective terms such as "tiny (infantile), small, medium, large, or very large." Wilson *et al.* (1970) devised a set of gauges covering the range of ovarian and uterine sizes; however, he found that palpation of the ovaries did not yield reliable information regarding reproductive events. Hartman (1932) and Mahoney (1970, 1972, 1975b) appear to be among the few who developed the skills required to obtain useful information by ovarian palpation of *M. mulatta* and *M. fascicularis.*

Diagnosing pregnancy by bimanual palpation is easier from approximately GD 16 onwards in macaques and baboons because of the relatively rapid changes that occur anatomically. Pregnancy is confirmed on the basis of size and consistency of the uterus until the 11th or 12th week (in macaques) at which time the head of the fetus can be palpated directly. Accurate diagnosis of pregnancy by uterine palpation can be accomplished by GD 25 in *M. mulatta* and by GD 20–21 in the baboon, providing the day(s) of mating is known. Abdominal palpation can also be utilized to diagnose pregnancy in New World species. In the squirrel monkey, this method is reliable by the 6th week of gestation when a 4- to 5-mm mass is detectable initially in the lower abdominal region cranial to the pelvis. This changes to a larger spongy mass by about 10–12 weeks (Kaplan, 1977). In the marmoset, estimates of uterine size based on external palpation have allowed formulation of an equation which permits prediction of parturition time (Gengozian *et al.*, 1974; Phillips and Grist, 1975). In the prosimians, abdominal palpation is the normal method of detection due to the dearth of information on the endocrinology of pregnancy in these species. For the greater galago, palpations are carried out weekly beginning 3 weeks from the estimated day of ovulation (Valerio *et al.*, 1972). Using this procedure, pregnancy can be accurately determined between 40 and 60 days gestation although earlier diagnosis is sometimes possible.

Wilson *et al.* (1970) developed a 24-hr biological test for pregnancy with a high predictive reliability. The test was performed by injecting immature mice weighing 8 to 12 g subcutaneously with maternal serum obtained between GD 15 and 32. Controls were equally treated with normal saline. The mice were euthanized 24 hr later in order to obtain uterine and body weights, and were used to calculate a uterine weight factor by the following formula:

$$\frac{\text{uterine weight (mg)}}{\text{body weight (g)}} \times 1000.$$

A uterine weight factor of 1.35 or greater was routinely accepted as a positive indication of pregnancy. A 90% or greater positive test could be obtained between GD 18 and 22 with pregnancy diagnosed more than 33–60% between GD 15 and 17 and 38–82% between GD 23 and 31. This test has been applied successfully in research colonies of *M. fascicularis* (Korte *et al.*, 1988).

Although it was known as early as 1955 that monkeys secrete chorionic gonadotropin (CG), quantitative assays were not available until 1966–1967, when Tullner and Hertz (1966) showed that CG appeared in measurable amounts as early as GD 15 and was detectable until approximately GD 30. The practical disadvantages of these methods were that (1) several days were required for completion of the tests, and (2) they involved time-consuming procedures such as augmentation by human CG.

Since this initial effort, a series of tests have been developed for diagnosing pregnancy. A hemagglutination inhibition test, which has become known as the NIH Subhuman Primate Pregnancy Test Kit (SHPT), was developed by Hodgen and Ross (1974) using an antiserum to the β subunit of ovine LH which is common to the CG of humans, gorillas, orangutans, chimpanzees, baboons, and macaques. This antiserum is, however, dissimilar to FSH and LH of baboons and macaques. Only 0.2 ml of neat urine (or an equivalent amount of urine extract) is required for testing, and results are obtained within 3 hr after the samples are collected. This method is reliable for the confirmation of pregnancy on GD 16 (fivefold concentration required) or by GD 18 (neat urine tested). Collection of aliquots of freshly voided urine is most satisfactory because it minimizes the time and collection of samples in addition to urine debris. The SHPT has become the method of choice for routine breeding management situations and has been used successfully in rhesus (Hodgen and Ross, 1974) and long-tailed macaques (Boot and Huis in't Veld, 1981), baboons (Hodgen and Niemann, 1975), and chimpanzees (Hodgen *et al.*, 1976). Both false-positive and false-negative tests are reported, due in part to the variability in urinary macaque CG excretion from animal to animal. This method of analyzing CG has also become a standard means for diagnosing pregnancy in many New World species. In the squir-

rel monkey, the concentrations of hormone gradually increase during early pregnancy and reach maximum values at midgestation (Diamond *et al.*, 1987). Analysis of CG is most accurate between 40 and 60 days of pregnancy; however, single determinations have an inherent 10% risk of false-negative responses due to low CG levels (Hodgen *et al.*, 1978). CG in the marmoset is excreted throughout pregnancy and maximum levels can be detected between the 8th and 9th week of gestation (Hearn *et al.*, 1988; Hobson *et al.*, 1977). Gestational levels of CG are first noted about 20 days after the LH peak in tamarins, and continues to be elevated for another 80 days (Hall and Hodgen, 1979; Heistermann *et al.*, 1987; Kleiman *et al.*, 1978; Ziegler *et al.*, 1987). In the owl monkey, CG can be detected about 16 weeks prepartum until birth.

Shaikh *et al.* (1976) found that pregnancy confirmation in baboons is more reliably detected by a plasma CG RIA than by the urine hemagglutination inhibition assay (Hodgen and Ross, 1974). Although this method is more time-consuming (results obtained 18 hr after sample collection), pregnancy is identified on GD 16 with 96.6% reliability. Plasma estradiol and progesterone RIA determinations, which can be obtained more quickly, have the same level of accuracy (96.6%) on GD 16 when evaluated according to a computer-derived formula.

Booher *et al.* (1983) developed a semiquantitative radioreceptor assay (RRA) for macaque LH/CG which was adapted from the clinical RRA for human LH/CG (Biocept-G) for the purposes of pregnancy detection prior to GD 20 as well as for estimating the time of ovulation. To perform the test, single blood samples were taken on GD 17–20. The assay (total time = 90 min) was performed according to the kit insert and analysis of positive and negative results is based on comparisons to reference control serum standards. Pregnancy was detected prior to GD 20 with 97.5% accuracy in both *M. mulatta* and *M. fascicularis*; false-negative results were observed at a rate of 2–3% and no false positives were recorded. This simple, accurate, and reliable test for early pregnancy was well suited for studies requiring diagnosis of pregnancy <GD 20; however, the cost of the Biocept-G pregnancy test kit (Wampole Labs, Cranbury, N.J.) precluded its continued use.

A quantitative RRA was employed for early diagnosis of pregnancy in *M. fascicularis* by determination of serum CG levels 3 to 4 weeks after conception (Yoshida *et al.*, 1987). Serum CG levels increased to 50 μg/ml in the majority of animals evaluated. Three weeks after conception, 86% of all pregnant animals showed a positive response and by 4 weeks after conception a 95% positive response was reported. Five percent of the tested animals yielded false negative responses at 4 weeks due to low CG levels; no false positive responses were reported.

A commercial human pregnancy test (Pregnostican; Organon, OSS, Holland) was employed to monitor urinary CG in the pregnant chimpanzee (Boorman *et al.*, 1974). Bimonthly screening of urine samples from breeding females indicated that this assay was a reliable method for detecting pregnancy in this species which secretes CG throughout most of pregnancy and for predicting parturition. CG production was found to be detectable for shorter periods in pregnancies that ended in spontaneous abortion.

Pregnancy monitoring via small urine volumes and measurements of immunoreactive E_t and LH/CG bioactivity have been accomplished successfully in four diverse species: the orangutan, pygmy chimpanzee, Douc langur, and capuchin (Czekala *et al.*, 1981). Urinary creatinine measurements are used to index all hormone concentrations. These studies show that measurement of E_t alone is sufficient to detect and monitor pregnancy in most species; in some species it may be necessary to assess individual estrogens if a more precise evaluation is necessary. The measurement of LH/CG bioactivity usually allows for earlier detection of pregnancy than E_t alone and provides additional information on implantation and placental function.

Direct RIA of urinary E_1C and progesterone metabolites is useful in the sequential monitoring of individual animals of the endangered species *M. silenus* (Shideler *et al.*, 1983). The applicability of these urinary measurements has been examined further in both *M. fascicularis* and *M. mulatta* (Monfort *et al.*, 1986, 1987). In *M. mulatta*, urinary E_1C profiles are both qualitatively and quantitatively similar to measurements of circulating estradiol. Inasmuch as urinary E_1C provides the same information as circulating serum estradiol, these measurements provide a noninvasive method that is practical for both prospective and retrospective longitudinal studies. These methods can also be applied to a variety of purposes including pregnancy diagnosis since it is a relatively simple and cost-effective assay.

Monitoring hormone metabolites in urine has also been used as a means of pregnancy detection in some New World species. Measurement of hydroxypregnenolone excretion has been used for this purpose in marmosets (Heger and Neubert, 1987; Hodges *et al.*, 1983), tamarins, and owl monkeys (Hall and Hodgen, 1979; Heistermann *et al.*, 1987; Kleiman *et al.*, 1978; Ziegler *et al.*, 1987).

The serum RIA used routinely at the CPRC for pregnancy detection is a fast and reliable method for early pregnancy detection in the macaque. It also utilizes human CG and is based on relative concentrations of macaque CG. The following steps are involved in the RIA: blood is collected by venipuncture (2 ml) from unanesthetized animals and is allowed to clot at room temperature. The blood is centrifuged, the serum is harvested, and is then either assayed immediately or stored at −20°C. The assay employs anti-bovine β-LH (Monoclonal Antibodies, Inc., Davis, CA) diluted 1:50,000 in monoclonal antibody buffer as the primary antibody. This relatively nonspecific antibody does not detect macaque LH at physiologic concentrations but is capable of detecting the rising levels of macaque CG approximately 2 weeks following implantation (Matteri *et al.*, 1987). Goat anti-mouse γ-globulin (IgG) (Monoclonal Antibodies, Inc., Davis, CA) diluted 1:200 in 5% polyethylene glycol buffer is the secondary antibody. The procedure involves the addition of iodinated (^{125}I) human CG (Diagnostic Products Corp., Los Angeles, CA) and normal mouse serum (Sigma Chemical Co.,

TABLE III

RADIOIMMUNOASSAY CHORIONIC GONADOTROPIN IN MACAQUE SERUM[a]

Macaque species	Number of tests (% of tests)				
	Total	True-positive	True-negative	False-positive	False-negative
Rhesus	1294	(193) 15%	(1075) 83%	0	(26) 2%
Long-tailed	919	(196) 21%	(703) 76%	0	(20) 2%

[a]Taken on Days 17–30 after estimated day of ovulation Sept. 1, 1987–Aug. 31, 1989 (two breeding seasons).

St. Louis, MO) to tubes containing monkey serum, control serum, or standard followed by a 1-hr incubation with the primary antibody at 4°C and a 15-min incubation with the secondary antibody at 4°C. Tubes are centrifuged at 3000–3500 rpm (800 *g*) for 20 min at 4°C, the supernatant is decanted, and the tubes are dried prior to counting the precipitate in a gamma counter. The values of macaque CG are calculated as the percent bound fraction determined by dividing the sample counts per minute (cpm) by the blank cpm. Pregnancy determination is based on the following criteria: positive result, <80% bound; negative result, >99% bound; and questionable result, 80–99% bound. All animals with questionable results are rebled in 2 to 3 days for final determination. Quantitative amounts of macaque CG are calculated using human CG standards (Calbiochem-Behring Corp., La Jolla, CA) ranging from 3 to 31 ng human CG/tube, respectively. The reliability of this assay has been verified by the low level of false-negative (2%) and the lack of false-positives over two macaque breeding seasons (Table III).

One of the more recent techniques employed for pregnancy detection and monitoring in macaques has been diagnostic ultrasound. This method provides a reliable means for evaluating and maintaining macaque reproductive colonies in addition to its application for experimental purposes. Animals may be hand held by experienced animal handlers, placed in restraint chairs (if previously trained), or immobilized with ketamine hydrochloride (10 mg/kg) for examinations. For pregnancy detection, the uterus is scanned transabdominally in both serial sagittal and transverse planes (Tarantal and Hendrickx, 1988a,d). Because of the characteristic thin abdominal wall in these species (*M. mulatta* and *M. fascicularis*), the high frequency transducers (i.e., 7.5 or 10 MHz) provide for optimal resolution and image quality. The uterus will usually be found midline, although anatomical variation and abdominal/pelvic adhesions can result in alternate locations. In many cases, the uterine body may be highly mobile and can be found flexed to the right, left, ventral (anteflexed), or dorsal (retroflexed) in relation to the cervix. This is frequently the case for females with an elongated lower uterine segment. In roughly 90% of all cases evaluated, a central linear echo is noted within the normal nongravid uterus. This echo represents the uterine "cavity"/interface between apposing layers of endometrium. The uterine or endometrial cavity echo (ECE) is a useful landmark for (1) identifying the uterus, (2) detecting early pregnancy, and (3) assessing uterine pathology.

Pregnancy can be identified in both the rhesus and long-tailed macaque as early as GD 14–16 (Tarantal and Hendrickx, 1988a). During this period of development the ECE shows a slight irregularity, thickening, or split in the upper one-third of the uterine body (Fig. 4). This represents the developing gestational sac (GS), which, by GD 18, appears as an ovoid fluid-filled structure approximately 5.5 ± 1.4 mm in mean diameter. Early pregnancy (GD 14/16–30) can be accurately detected and assessed using the sonographic developmental guidelines previously established (see Table IV). These guidelines are useful for assessing early pregnancies at risk, provided that normal developmental variation and methods for mating are kept in mind. In addition, no false positives or negatives result when incorporating these techniques (Tarantal and Hendrickx, 1988d). Once pregnancy is identified, a variety of measurements [mean GS size, yolk sac diameter, greatest length (GL) of the embryo, and heart rate] are important for monitoring normal development (Tarantal and Hendrickx, 1988a,b,c) (Table V).

Of interest is the sonographic appearance of "implantation bleeding" (placental sign; see Chapter 9). Hypoechoic areas may appear to surround the developing GS or within discrete regions such as cranial and caudal to the GS. This can be distinguished from early signs of threatened or impending abortion where intrauterine hemorrhage may be associated with hematoma formation. Both can result in extensive vaginal hemorrhage. Early resorptions can also be identified by an inappropriate GS size for the expected GD, poor development of the chorionic villi/placenta, or a diminishing GS when examined consecutively. A small volume of intrauterine fluid within an enlarged uterus is also suggestive of early abortion/resorption, although small volumes of intrauterine fluid may be observed in some nongravid animals during the follicular phase of the menstrual cycle.

As discussed in Chapter 9, the incidence of live-born twins in Old World monkeys is extremely rare (Tarantal and Hendrickx, 1988d). Compilation of the published data for the rhesus indicates a twinning rate of 0.2% (10/4991). However, the appearance of twin GS, one with a viable embryo and one anembryonic, has been noted sonographically, which suggests that the incidence of multiple gestations is probably greater than reported. These data are similar to the "vanishing twin" phenomena as noted in the human, although the outcome in the macaque appears less favorable.

For those females determined nonpregnant, recording baseline data of uterine size and appearance has been suggested (Tarantal and Hendrickx, 1988d). These include measurements of total uterine length [uterine body and cervix, normal range for *M. mulatta* (Mm) is 52.0 ± 7.1 mm; *M. fascicularis* (Mf) is 43.6 ± 9.2 mm]; uterine body length (Mm 28.2 ± 5.0 mm; Mf

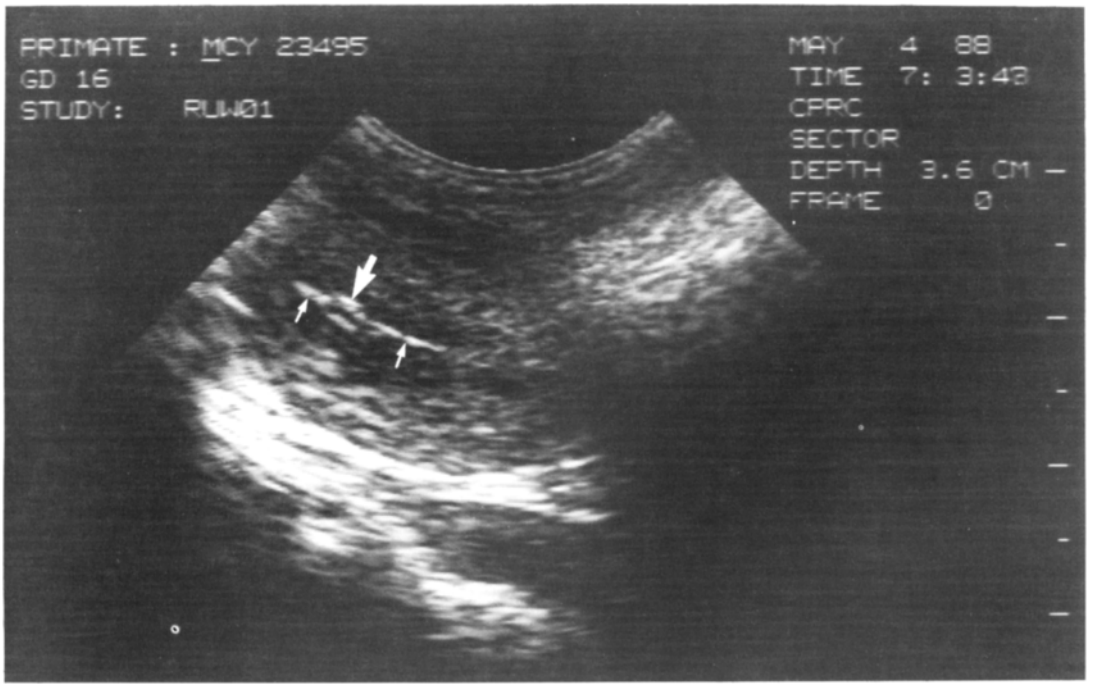

Fig. 4. Sonogram (sagittal section) of macaque uterus on Gestational Day (GD) 16. Note endometrial (uterine) cavity echo (small arrows) and developing gestational sac (large arrow).

23.7 ± 4.3 mm), width (Mm 17.8 ± 3.4 mm; Mf 16.8 ± 2.8 mm), and height (Mm 17.5 ± 3.4 mm; Mf 16.3 ± 2.7 mm); uterine shape, contour, and homogeneity; appearance of the ECE; and endometrial thickness. This information is particularly useful for documenting early absortions/resorptions and for evaluating nonreproductive females. Alterations in uterine size, contour, and/or appearance (i.e., changes in echogenicity) may be suggestive of a pathological process such as leiomyoma, carcinoma, adenomyosis, endometritis, or endometrial hyperplasia. Foreign bodies and seminal plugs/coagulum can be readily identified within the vaginal canal, fornices, or cervix/endocervical canal by an increase in echogenicity (hyperechoic) and, in some cases, acoustic shadowing. Of particular important is the detection of endometriosis, a relatively frequent finding in laboratory-housed macaques with a history of repeat hysterotomies. Endometriomas may occur in single or multiple sites attached to the uterus and/or adnexal structures, either homogeneous or septated, and will usually appear cystic with well-defined borders and some internal echoes. Diagnosis can be confirmed by ultrasound-guided aspiration of "chocolate fluid," which is a characteristic feature. In some cases, endometriomas may be complex in appearance (cystic and solid components) or predominantly solid, if of long-standing duration. Other types of cystic structures can be imaged in the mesentery (common; 1–10 mm in length; benign), ovary (may be follicles or thecalutein cysts), uterus (hydro-, pyo- or hematometra), or cervix (nabothian cysts) which can be aspirated, if deemed appropriate. Other pelvic and/or uterine solid neoplasms can be detected and biopsied to confirm the diagnosis.

The use of ultrasound-guided interventive procedures in these species has been described and its application to both the pregnant (Tarantal, 1990) and nonpregnant female (Tarantal *et al.*, 1990) established. The primary advantage of these techniques is the elimination for the need of extensive surgical procedures with all the associated risk, cost, and potential trauma.

Ultrasound has also been used to diagnose pregnancy and monitor fetal growth in squirrel monkeys (Narita *et al.*, 1988). Pregnancy can be identified on GD 25 with detection of the GS; cardiac activity can be confirmed approximately 2 weeks later.

TABLE IV

SONOGRAPHIC DEVELOPMENTAL GUIDELINES[a]

Feature	GD
Thickening and/or split in ECE	14–15
Gestational sac	16–18
Yolk sac	18–20
Embryo	21–25
Cardiac motion	21–25

[a]Gestational days (GD) 14–25. ECE, endometrial cavity echo.

TABLE V

MEAN GESTATIONAL SAC SIZE (GS), YOLK SAC DIAMETER (YS), GREATEST LENGTH (GL), AND EMBRYONIC HEART RATES (EHR) FOR BOTH RHESUS AND LONG-TAILED MACAQUE[a]

GD	GS (mm)	YS (mm; range)	GL (mm)	EHR (bpm)
14	1.9 ± 0.2	—	—	—
15	2.6 ± 0.5	—	—	—
16	2.3 ± 0.4	—	—	—
17	3.7 ± 1.0	—	—	—
18	5.5 ± 1.4	1–2	—	—
19	5.9 ± 2.4	1–2	—	—
20	7.0 ± 2.6	2–3	—	—
21	8.9 ± 2.8	2–3	2.0 ± 0.0	60–80
22	9.3 ± 2.9	2–3	2.9 ± 0.6	72–104
23	10.7 ± 2.3	3	3.4 ± 0.8	80–120
24	12.4 ± 3.3	2–3	4.7 ± 0.9	80–144
25	13.0 ± 3.4	2–4	4.9 ± 0.9	92–144
26	13.5 ± 3.3	2–5	5.4 ± 1.9	100–140
27	14.9 ± 3.4	3–4	6.5 ± 1.5	100–144
28	16.0 ± 3.2	3–4	6.5 ± 1.8	108–148
29	18.1 ± 3.9	2–4	7.9 ± 1.5	120–152
30	18.3 ± 3.6	2–4	8.9 ± 1.7	120–156
31	20.7 ± 3.9	3–4	8.8 ± 1.9	128–160
32	19.9 ± 4.0	3–4	10.5 ± 2.3	132–160
33	23.0 ± 3.6	3–5	12.0 ± 2.1	128–160
34	24.2 ± 4.7	3–5	12.9 ± 2.6	128–160
35	24.2 ± 3.5	3–5	14.0 ± 2.7	124–168
36	28.1 ± 3.9	4–5	15.6 ± 2.0	124–156
37	27.0 ± 3.5	4–5	16.3 ± 1.9	144–176
38	29.2 ± 3.6	4–5	17.2 ± 1.9	140–184
39	28.1 ± 5.1	4–5	18.1 ± 2.1	132–184
40	31.9 ± 3.8	4–5	18.7 ± 2.0	132–168
41	32.2 ± 3.4	4–6	20.4 ± 1.9	140–180
42	32.6 ± 3.3	4–5	21.4 ± 2.1	140–172
43	33.2 ± 3.7	4–7	24.2 ± 2.9	144–172
44	34.4 ± 3.4	4–9	24.4 ± 2.2	144–176
45	33.4 ± 3.8	4–9	26.6 ± 3.7	144–180
46	37.2 ± 3.4	6–9	27.3 ± 3.1	148–184
47	38.4 ± 2.6	5–9	28.7 ± 4.7	144–180

[a]*Macaca mulatta, Macaca fascicularis.* Gestational days (GD) 14–47. Both species similar in size during organogenesis (see Tarantal and Hendrickx, 1988a,b).

GS measured in three dimensions and mean value calculated. GL measured from head to base of tail (see Tarantal and Hendrickx, 1988b).

No differences in uterine size between pregnant and nonpregnant animals have been observed 135 days prior to delivery.

VI. PRENATAL GROWTH AND DEVELOPMENT

Routine observations for evaluating prenatal growth sonographically at the CPRC (*M. mulatta* and *M. fascicularis*) include mean GS dimension (GD 14–50), GL (~GD 23–60), head measurements (biparietal and occipitofrontal diameters, head area, and circumference), abdominal area and circumference, and humerus and femur lengths (see Tarantal and Hendrickx, 1988b,c, for methods). These measurements are compared to normative growth data or predicted values (Tables VI–VIII) for each species and are used in combination to obtain greater accuracy for either predicting or confirming gestational age (Tarantal and Hendrickx, 1988b). In addition, embryonic/fetal heart rates can be obtained during the examination period and compared to the normal rates expected.

Documentation of normal and abnormal growth and development of the conceptus is particularly pertinent, both from a colony maintenance standpoint and for research-oriented purposes. The standard sonographic evaluation performed during the second trimester (~GD 75–90) is incorporated in order to assess anatomical configuration, determine gender, monitor condition, and evaluate placental development. This is the optimal time for making judgments regarding conformation of the fetus since the volume of amniotic fluid (i.e., the ratio of fetus to the fluid volume) provides an excellent sonographic "window." Development of the brain (i.e., lateral, third, and fourth ventricles; thalamus; midbrain; cerebellum; cerebral hemispheres; choroid plexus; cranial base), face (eyes, nose,

TABLE VI

PREDICTED VALUES FOR GESTATIONAL SAC (GS) AND GREATEST LENGTH (GL) FOR THE RHESUS AND LONG-TAILED MACAQUE (*M. MULATTA* AND *M. FASCICULARIS*)

GD	GS (mm)	GL (mm)	GD	GS (mm)	GL (mm)
15	1.4	—	38	28.7	16.9
16	2.7	—	39	29.6	18.2
17	3.9	—	40	30.7	19.5
18	5.1	—	41	31.7	20.9
19	6.3	—	42	32.8	22.4
20	7.5	—	43	33.9	23.9
21	8.7	2.9	44	34.9	25.5
22	10.0	3.3	45	36.0	27.1
23	11.2	3.8	46	37.0	28.8
24	12.3	4.3	47	38.0	30.5
25	13.5	4.9	48	39.0	32.3
26	14.7	5.5	49	40.1	34.2
27	15.9	6.2	50	41.1	36.1
28	17.1	6.9	51	—	38.1
29	18.3	7.7	52	—	40.1
30	19.4	8.5	53	—	42.2
31	20.6	9.4	54	—	44.4
32	21.7	10.3	55	—	46.7
33	22.9	11.3	56	—	49.0
34	24.0	12.3	57	—	51.3
35	25.1	13.4	58	—	53.8
36	26.2	14.5	59	—	56.3
37	27.4	15.7	60	—	58.8

Note. GS and GL are measured as described under Table V. During early gestation (GD 14–25), GS used for predicted gestational age (pGA); for GD > 25–60, GL used for pGA (see Tarantal and Hendrickx, 1988b).

TABLE VII

PREDICTED VALUES FOR BIPARIETAL DIAMETER (BPD) FOR THE RHESUS (MM) AND LONG-TAILED (MF) MACAQUE[a]

GD	Mm	Mf	GD	Mm	Mf	GD	Mm	Mf
47	10.90	10.91	87	30.14	29.42	127	43.35	41.39
48	11.44	11.44	88	30.55	29.81	128	43.58	41.59
49	11.97	11.96	89	30.96	30.20	129	43.81	41.78
50	12.50	12.48	90	31.36	30.58	130	44.03	41.96
51	13.03	13.00	91	31.76	30.95	131	44.25	42.14
52	13.56	13.51	92	32.16	31.32	132	44.47	42.32
53	14.09	14.02	93	32.55	31.69	133	44.68	42.49
54	14.61	14.53	94	32.94	32.05	134	44.88	42.65
55	15.13	15.03	95	33.33	32.41	135	45.08	42.81
56	15.64	15.54	96	33.71	32.76	136	45.27	42.96
57	16.16	16.03	97	34.09	33.11	137	45.46	43.10
58	16.67	16.53	98	34.46	33.46	138	45.64	43.24
59	17.18	17.02	99	34.83	33.80	139	45.82	43.38
60	17.68	17.51	100	35.19	34.14	140	45.99	43.51
61	18.19	18.00	101	35.55	34.47	141	46.15	43.63
62	18.69	18.48	102	35.91	34.80	142	46.31	43.75
63	19.18	18.96	103	36.26	35.12	143	46.47	43.86
64	19.68	19.44	104	36.61	35.44	144	46.62	43.96
65	20.17	19.91	105	36.95	35.75	145	46.76	44.06
66	20.66	20.38	106	37.29	36.06	146	46.90	44.15
67	21.13	20.85	107	37.63	36.36	147	47.03	44.24
68	21.62	21.31	108	37.96	36.66	148	47.16	44.32
69	22.10	21.77	109	38.28	36.96	149	47.28	44.39
70	22.58	22.23	110	38.60	37.25	150	47.39	44.46
71	23.05	22.68	111	38.92	37.53	151	47.50	44.52
72	23.52	23.13	112	39.23	37.81	152	47.60	44.57
73	23.98	23.58	113	39.54	38.08	153	47.70	44.62
74	24.44	24.02	114	39.84	38.35	154	47.79	44.67
75	24.90	24.46	115	40.14	38.62	155	47.87	44.70
76	25.36	24.89	116	40.44	38.88	156	47.95	—
77	25.81	25.32	117	40.72	39.13	157	48.03	—
78	26.26	25.75	118	41.01	39.38	158	48.09	—
79	26.70	26.17	119	41.29	39.63	159	48.15	—
80	27.15	26.59	120	41.56	39.87	160	48.21	—
81	27.58	27.01	121	41.83	40.10	161	48.26	—
82	28.02	27.42	122	42.10	40.33	162	48.30	—
83	28.45	27.83	123	42.36	40.55	163	48.33	—
84	28.88	28.23	124	42.61	40.77	164	48.36	—
85	29.30	28.64	125	42.86	40.98	165	48.39	—
86	29.72	29.03	126	43.11	41.19			

Note. For GD 50–60, use GL and BPD for pGA; for GD >60, use BPD and FL for pGA. See Tarantal and Hendrickx (1988b) for a description of methods for obtaining measurements, techniques for use, and accuracy/reliability.

[a]Gestational Days (GD) 47–165. Used in combination with femur length (FL; see Table VIII) to confirm/predict gestational age (pGA).

TABLE VIII

PREDICTED VALUES FOR FEMUR LENGTH (FL) FOR THE RHESUS (MM) AND LONG-TAILED (MF) MACAQUE[a]

GD	Mm	Mf	GD	Mm	Mf	GD	Mm	Mf
50	2.61	2.84	89	19.05	18.84	128	34.48	32.08
51	3.00	3.27	90	19.48	19.22	129	34.81	32.37
52	3.40	3.70	91	19.91	19.60	130	35.15	32.65
53	3.79	4.13	92	20.33	19.97	131	35.48	32.94
54	4.19	4.56	93	20.76	20.35	132	35.80	33.22
55	4.60	4.99	94	21.19	20.72	133	36.13	33.49
56	5.00	5.42	95	21.61	21.09	134	36.44	33.77
57	5.41	5.84	96	22.04	21.46	135	36.76	34.04
58	5.82	6.27	97	22.46	21.83	136	37.06	34.31
59	6.23	6.69	98	22.88	22.20	137	37.37	34.57
60	6.64	7.12	99	23.30	22.56	138	37.66	34.83
61	7.06	7.54	100	23.72	22.92	139	37.96	35.09
62	7.47	7.96	101	24.13	23.28	140	38.25	35.35
63	7.89	8.38	102	24.55	23.64	141	38.53	35.60
64	8.31	8.80	103	24.96	23.99	142	38.81	35.85
65	8.73	9.22	104	25.37	24.34	143	39.08	36.10
66	9.15	9.63	105	25.78	24.70	144	39.35	36.34
67	9.58	10.05	106	26.19	25.04	145	39.62	36.58
68	10.00	10.46	107	26.60	25.39	146	39.87	36.81
69	10.43	10.88	108	27.00	25.73	147	40.13	37.05
70	10.85	11.29	109	27.40	26.07	148	40.37	37.27
71	11.28	11.70	110	27.80	26.41	149	40.62	37.50
72	11.71	12.11	111	28.20	26.75	150	40.85	37.72
73	12.14	12.52	112	28.59	27.08	151	41.08	37.94
74	12.57	12.92	113	28.98	27.42	152	41.30	38.16
75	13.00	13.33	114	29.37	27.74	153	41.52	38.37
76	13.43	13.73	115	29.75	28.07	154	41.73	38.58
77	13.87	14.13	116	30.14	28.40	155	41.94	38.78
78	14.30	14.53	117	30.52	28.72	156	42.14	—
79	14.73	14.93	118	30.89	29.04	157	42.33	—
80	15.16	15.33	119	31.27	29.35	158	42.52	—
81	15.59	15.73	120	31.64	29.67	159	42.70	—
82	16.03	16.12	121	32.00	29.98	160	42.87	—
83	16.46	16.52	122	32.37	30.29	161	43.04	—
84	16.89	16.91	123	32.73	30.59	162	43.20	—
85	17.32	17.30	124	33.09	30.90	163	43.36	—
86	17.76	17.68	125	33.44	31.19	164	43.50	—
87	18.19	18.07	126	33.79	31.49	165	43.64	—
88	18.62	18.45	127	34.13	31.79			

Note. For GD >60, use BPD and FL for pGA. See Tarantal and Hendrickx (1988b) for a description of methods for obtaining measurements, techniques for use, and accuracy/reliability.

[a]Gestational Days (GD) 50–165. Used in combination with biparietal diameter (BPD; see Table VII) to confirm/predict gestational age (pGA).

mouth), heart, abdominal viscera, and axial and appendicular skeleton can all be assessed with accuracy. In addition, placental location (mono- versus bidiscoid) and development (aging changes) are particularly important, especially when monitoring for placenta previa or placental abruptions. Those animals documented with either a marginal or a complete previa during the second trimester can be reevaluated later in gestation and scheduled for cesarean section, as required. It is important to rescan during the latter stages of development as the placenta may "migrate" (i.e., as the uterus grows the discs are displaced craniad). Diagnosis of this condition requires accurate localization of the placenta in relation to the cervix on longitudinal scans. It should be noted that a distended urinary bladder may alter the relationship of the placenta to the cervix which can

lead to misinterpretation. Animals that display retroplacental or subchorionic hemorrhage are also closely monitored; if continued and considerable hemorrhage is noted, emergency surgery is performed. Of the nine concealed abruptions detected during three breeding seasons (GD 51–125; 9/873 or 1%), no maternal deaths occurred. One interesting feature that has been frequently associated with abruptions is the proliferation of decidual particularly near the lower uterine segment (Tarantal and Hendrickx, 1988d). Continued surveillance within the colony for repeat incidence has resulted in the removal of females at risk with a resultant decrease in occurrence.

The use of a modified biophysical profile (BPP) as performed in the human fetus has been incorporated with a variety of observations related to fetal activity *in utero*. Similar to the human, the nonhuman primate fetus is very active *in utero*, particularly during the early fetal period (GD 50–70) (Tarantal and Hendrickx, 1988d). By GD 80–100, vigorous whole body movements are less frequently observed and more selective activities such as darting eye movements, oral activities, and extension and flexion of the limbs and head may be noted. The BPP has proven useful for evaluating fetal status and well-being during the third trimester in unanesthetized dams (chair-restrained; A. F. Tarantal and M. S. Golub, unpublished observations) primarily for experimental purposes. An observation period of 20 min on GD 115, 125, 135, and 145 includes documentation of changes in fetal heart rate and quantitation of respiratory and motor activity, muscle tone, and whole-body startle.

A comprehensive overview of the many studies and methods for analyzing growth of the nonhuman primate fetus, including body and organ weights and dimensional and proportional growth, has been provided by Brizzee and Dunlap (1986) and will not be repeated herein. Readers are referred to this review for further information.

VII. MATERNAL CHANGES WITH PREGNANCY

In nonpregnant *S. sciureus*, daily water consumption ranges from 20 to 160 ml (mean 110 ml) which increases to 346 ml per day during the fifth month of pregnancy. A significant decrease to nonpregnant levels has been noted 2 to 16 days prior to delivery (Clewe, 1969). Travis and Holmes (1974) have reported a linear increase in water consumption from the day of conception through GD 138, with a correlative significant increase in mean daily urine output. Evaluation of four species of nonhuman primates (*M. mulatta, M. speciosa, E. patas*, and *P. troglodytes*) has shown that significant upper ureteral dilatation occurs during pregnancy, which is similar to the human (Roberts and Wolf, 1971).

An increase in plasma volume in comparison to red cell mass occurs in primates throughout gestation. This hydremia of pregnancy is important in maintaining the health of the fetus by ensuring adequate uterine perfusion. An approximate 30% increase in blood volume has been reported for *M. mulatta* (Allen and Ahlgren, 1968) with increases in total volumes of red and white blood cells (WBCs), hemoglobin, total plasma protein, and albumin, which decrease substantially at parturition. Other studies have indicated a shift in the albumin:globulin ratio and an increase in the sedimentation rate and plasma fibrinogen (Allen and Siegfried, 1966; Knapp *et al.*, 1974). A neutrophilia has also been observed beginning ~GD 50 (Allen and Siegfried, 1966). Studies by Spicer and Oxnard (1967) have also shown a decrease in hemoglobin late in pregnancy, which was well-correlated with reductions in mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and iron, and an increase in mean corpuscular volume. A further demonstration of changes both pre- and postpartum has been provided by Switzer *et al.* (1970); packed cell volumes decreased during the first trimester (attributed to implantation bleeding) and again 72–96 hr postdelivery. Sedimentation rates also increased during the third trimester with a peak 72 hr postpartum. Interestingly, WBCs decreased midgestation with a reversed ratio of lymphocytes:neutrophils during the second trimester; eosinophils also decreased during the third trimester with lowest values detected at parturition.

Reference values for hematologic parameters and clinical chemistry screens from the CPRC rhesus (GD 45, 90, 135, 165; $N = 13$) and long-tailed (GD 25, 50, 75, 100; $N = 10$) macaque colonies are presented in Tables IX–XII. For both species, samples were collected from unanesthetized females in their cages (extension of an arm or leg out of a partially opened door). In contrast to the literature just cited, only marginal changes in all parameters were observed. These differences may be attributed to the methods used for collection and the frequency of sampling. For clinical chemistry screens, rhesus showed a minor increase in blood urea nitrogen (BUN), glucose, and alkaline phosphatase (ALP), with a decraese in albumin and total proteins at term. Marginal decreases were also observed for carbon dioxide, potassium, and γ-glutamyltransferase. On GD 100, the long-tailed macaques showed reductions in BUN, total proteins, and ALP.

Of interest are studies performed in *M. nemestrina* which specifically addressed the effects of pregnancy on high density lipoproteins (HDL) concentrations. Although HDL decrease (Schiller *et al.*, 1983), increases in low density lipoproteins (LDL) were noted in late pregnancy (Rudel *et al.*, 1981). In addition, levels of HDL were predictive of the pregnancy outcome since no changes in HDL were observed in pregnancies that resulted in spontaneous abortion (Schiller *et al.*, 1983). It was hypothesized that this lack of a decrease may be attributed to fetal–placental dysfunction (i.e., decreased HDL-cholesterol utilization for steroid biosynthesis).

The average total weight gain during pregnancy for *M. nemestrina* has been reported to be 19% above the preconception weight (Goodlin and Sackett, 1983). It was also observed that individual animals lost from 5 to 10% of their mean preconception weight during the first 45 days of pregnancy. By GD 60, a

TABLE IX

HEMATOLOGY REFERENCE VALUES FOR *M. MULATTA*[a]

Parameter	GD 45	(Range)	GD 90	(Range)	GD 135	(Range)	GD 165	(Range)
RBC ($\times 10^6/\mu l$)	5.6 ± 0.5	(4.7–6.4)	5.6 ± 0.5	(4.9–6.2)	5.4 ± 0.5	(4.5–6.3)	4.7 ± 0.7	(3.9–6.3)
HgB (g/dl)	21.7 ± 1.0	(10.2–14.1)	12.9 ± 0.7	(11.7–13.9)	12.7 ± 0.9	(11.4–14.4)	11.2 ± 1.6	(9.5–14.5)
HCT (%)	38.9 ± 3.2	(31.2–44.7)	39.9 ± 2.4	(36.8–43.0)	38.4 ± 3.0	(33.8–43.9)	33.3 ± 5.0	(27.4–43.7)
MCV (fl)	69.9 ± 3.3	(65–73)	71.8 ± 2.9	(67–77)	71.5 ± 2.4	(68–76)	71.2 ± 3.0	(67–76)
MCH (pg)	22.9 ± 1.1	(21.0–25.0)	23.3 ± 1.1	(21.5–25.6)	23.6 ± 1.1	(21.5–25.2)	24.0 ± 1.3	(22.1–26.3)
MCHC (pg/fl)	32.7 ± 0.9	(31.5–35.1)	32.5 ± 0.8	(31.4–33.4)	32.9 ± 0.7	(31.5–34.2)	33.7 ± 0.8	(32.6–34.8)
PP (g/dl)	7.2 ± 0.3	(6.7–7.8)	6.8 ± 0.4	(6.3–7.8)	6.9 ± 0.3	(6.2–7.4)	6.6 ± 0.4	(5.8–7.2)
Fibrin. (mg/dl)	192 ± 64	(<100–300)	192 ± 86	(<100–400)	154 ± 52	(100–200)	277 ± 101	(100–500)
WBC ($\times 10^3/\mu l$)	8.0 ± 2.4	(4.7–13.4)	8.8 ± 2.8	(5.1–14.7)	7.9 ± 2.1	(4.8–11.0)	7.9 ± 2.0	(4.8–12.6)
Seg. Neutr. (%)	52.8 ± 11.2	(33–68)	58.9 ± 11.9	(41–77)	61.8 ± 10.6	(45–85)	70.2 ± 8.4	(58–82)
(/μl)	4,331 ± 1,819	(1974–8040)	5384 ± 2634	(2142–11,319)	4987 ± 1861	(2208–8670)	5566 ± 1589	(3264–8820)
Lymph. (%)	42.5 ± 11.5	(28–65)	35.5 ± 12.7	(18–55)	31.2 ± 9.1	(13–45)	25.5 ± 7.3	(14–39)
(/μl)	3,288 ± 966	(2175–4947)	2891 ± 778	(1406–4550)	2410 ± 822	(1326–3630)	1972 ± 574	(1050–2772)
Mon. (%)	3.3 ± 2.1	(1–7)	4.0 ± 2.0	(1–8)	5.2 ± 2.6	(1–10)	3.8 ± 2.5	(1–8)
(/μl)	262 ± 218	(0–670)	321 ± 214	(0–630)	374 ± 141	(75–546)	312 ± 275	(57–1008)
Eos. (%)	2.9 ± 1.2	(1–4)	2.4 ± 1.0	(1–4)	2.3 ± 0.9	(0–4)	2.0 ± 0.0	(0–2)
(/μl)	137 ± 179	(0–536)	158 ± 167	(0–504)	137 ± 105	(0–312)	30 ± 58	(0–148)
Baso. (%)	1.0 ± 0.0	(0–1)	1.0 ± 0.0	(0–1)	1.0 ± 0.0	(0–1)	1.0 ± 0.0	(0–1)
(/μl)	13 ± 32	(0–103)	15 ± 42	(0–147)	7.3 ± 26.4	(0–95)	6 ± 20	(0–72)

Note. RBC, red blood cells; HgB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PP, plasma protein; Fibrin, fibrinogen; WBC, white blood cells; Seg. Neutr., segmented neutrophils; Lymph., lymphocytes; Mono., monocytes; Eos., eosinophils; Baso., basophils.

[a]Gestational Days (GD) 45–165 (N = 13; mean ± SD).

TABLE X

HEMATOLOGY REFERENCE VALUES FOR *M. FASCICULARIS*[a]

Parameter	GD 25	(Range)	GD 50	(Range)	GD 75	(Range)	GD 100	(Range)
RBC ($\times 10^6/\mu l$)	5.7 ± 0.6	(4.8–7.0)	6.0 ± 0.5	(5.2–6.4)	6.4 ± 0.5	(5.7–7.2)	6.4 ± 0.5	(5.6–7.0)
HgB (g/dl)	10.8 ± 0.8	(9.5–12.1)	11.7 ± 0.6	(10.5–12.3)	12.4 ± 0.9	(11.5–14.2)	12.2 ± 0.8	(11.4–13.4)
HCT (%)	36.3 ± 2.8	(31.4–41.8)	37.8 ± 2.7	(32.4–40.4)	40.4 ± 2.8	(37.2–45.4)	40.2 ± 2.1	(37.3–43.1)
MCV (fl)	64 ± 2	(59–66)	64 ± 2	(59–67)	63 ± 2	(60–67)	63 ± 3	(58–67)
MCH (pg)	19.0 ± 1.1	(17.2–20.4)	19.7 ± 0.8	(18.5–20.2)	19.3 ± 0.9	(18.1–20.7)	19.1 ± 0.9	(17.6–20.2)
MCHC (pg/fl)	29.9 ± 0.9	(28.7–31.5)	31.0 ± 0.9	(29.7–32.4)	30.6 ± 1.2	(28.7–32.2)	30.4 ± 0.9	(28.6–31.7)
WBC ($\times 10^3/\mu l$)	8.1 ± 1.6	(5.9–10.2)	8.0 ± 1.5	(6.4–11.1)	7.6 ± 1.8	(4.3–11.0)	7.9 ± 1.6	(6.3–10.7)
Seg. Neutr. (%)	53 ± 10	(40–71)	58 ± 6	(47–68)	58 ± 6	(50–66)	65 ± 11	(52–83)
(/μl)	4243 ± 872	(2542–5429)	4597 ± 797	(3584–6380)	4400 ± 1058	(2709–6283)	5138 ± 1,512	(3816–8881)
Lymph. (%)	40 ± 8	(22–48)	36 ± 7	(29–50)	35 ± 6	(29–46)	30 ± 9	(16–43)
(/μl)	3280 ± 1083	(1298–4896)	2876 ± 775	(1856–4150)	2719 ± 806	(1419–3818)	2402 ± 947	(1188–4429)
Mono. (%)	5 ± 3	(2–12)	4 ± 3	(1–10)	6 ± 3	(2–12)	4 ± 3	(0–10)
(/μl)	445 ± 314	(142–1224)	360 ± 290	(83–790)	441 ± 281	(150–1080)	312 ± 241	(0–760)
Eos. (%)	2 ± 2	(0–6)	1 ± 2	(0–7)	1 ± 1	(0–2)	1 ± 1	(0–4)
(/μl)	132 ± 139	(0–378)	123 ± 192	(0–588)	51 ± 52	(0–130)	80 ± 101	(0–272)
Baso. (%)	0 ± 0	(0–1)	0 ± 0	(0)	0 ± 0	(0–1)	0 ± 0	(0–1)
(/μl)	19 ± 41	(0–99)	0 ± 0	(0)	21 ± 43	(0–103)	0 ± 0	(0)

Note. RBC, red blood cells; HgB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PP, plasma protein; Fibrin., fibrinogen; WBC, white blood cells; Seg. Neutr., segmented neutrophils; Lymph., lymphocytes; Mono., monocytes; Eos., eosinophils; Baso., basophils.

[a]Gestational Days (GD) 25–100 (N = 10; mean ± SD).

TABLE XI

CLINICAL CHEMISTRY REFERENCE VALUES FOR *M. MULATTA*[a]

Parameter	GD 45	(Range)	GD 90	(Range)	GD 135	(Range)	GD 165	(Range)
Cl (m*M*/liter)	107.8 ± 2.1	(104–111)	108.8 ± 2.2	(106–114)	109.8 ± 1.7	(107–112)	111.3 ± 1.7	(109–116)
TCO_2 (m*M*/liter)	23.6 ± 5.0	(16–37)	25.0 ± 4.0	(19–36)	21.6 ± 2.3	(17–25)	22.7 ± 2.5	(18–26
K (m*M*/liter)	4.6 ± 0.5	(4.0–5.8)	4.8 ± 0.3	(4.1–5.3)	4.8 ± 0.4	(4.3–5.6)	4.0 ± 0.3	(3.6–4.7)
Na (m*M*/liter)	144.1 ± 1.7	(142–149)	143.5 ± 1.9	(140–146)	144.2 ± 1.8	(141–148)	147.0 ± 2.7	(142–153)
AG (m*M*/liter)	17.3 ± 4.5	(7–26)	14.5 ± 4.2	(4–21)	17.8 ± 3.7	(12–26)	17.1 ± 2.8	(13–22)
Alb (g/dl)	3.9 ± 0.4	(3.1–4.8)	3.4 ± 0.5	(2.9–4.6)	3.2 ± 0.3	(2.6–3.5)	2.6 ± 0.3	(2.1–3.1)
BUN (mg/dl)	13.2 ± 1.6	(11–16)	12.1 ± 1.8	(9–15)	12.0 ± 1.7	(8–14)	23.5 ± 2.9	(20–29)
Glucose (mg/dl)	50.4 ± 5.7	(44–64)	47.2 ± 8.5	(38–70)	52.4 ± 11.6	(22–67)	58.7 ± 23.4	(39–95)
TP (g/dl)	7.4 ± 0.6	(6.7–8.8)	6.9 ± 0.5	(6.0–7.8)	7.0 ± 0.3	(6.5–7.5)	5.9 ± 1.1	(2.7–7.1)
ALT (U/liter)	32.5 ± 13.3	(17–69)	34.5 ± 12.3	(15–58)	30.7 ± 16.9	(12–66)	30.8 ± 17.6	(11–74)
ALP (U/liter)	111.2 ± 43.1	(26–196)	96.5 ± 53.2	(18–232)	135.1 ± 62.1	(35–268)	138.4 ± 68.6	(40–281)
Ca (mg/dl)	9.8 ± 0.5	(9.1–10.6)	9.4 ± 0.3	(8.8–9.9)	9.3 ± 0.3	(8.7–9.9)	9.3 ± 0.4	(8.7–9.8)
Cr (mg/dl)	0.9 ± 0.1	(0.7–1.1)	0.8 ± 0.1	(0.7–0.9)	0.8 ± 0.1	(0.6–1.1)	0.9 ± 0.1	(0.7–1.1)
P (mg/dl)	3.9 ± 0.5	(3.4–4.8)	3.8 ± 0.7	(2.9–5.3)	4.0 ± 0.5	(3.5–5.6)	3.8 ± 0.6	(2.6–4.6)
γGT (U/liter)	41.2 ± 7.0	(31–58)	50.9 ± 13.0	(30–71)	49.7 ± 18.6	(25–104)	39.2 ± 8.8	(25–53)

Note. Cl, chloride; TCO_2, total carbon dioxide; K, potassium; Na, sodium; AG, anion gap; Alb, albumin; BUN, blood urea nitrogen; TP, total protein; ALT, alanine aminotransferase; ALP, alkaline phosphatase; Ca, calcium; Cr, creatinine; P, phosphorus; γGT, γ-glutamyltransferase.

[a]Gestational days (GD) 45–165 (N = 13; mean ± SD).

body weight gain was initiated which peaked at roughly GD 160. Maternal body weight changes during pregnancy from the CPRC rhesus and long-tailed macaque colonies are shown in Fig. 5. Rhesus data were collected periodically during gestation (GD 60, 90, 120, 150, and postpartum) from females participating in chair-restrained blood pressure monitoring beginning on GD 90. Long-tailed macaque body weights were collected from sham controls (GD 20 to >150) hand-caught for oral gavage during GD 20–50. Similar to observations in *M. nemestrina*, a decline in body weight was observed prior to GD 50 in the long-tailed macaque.

VIII. PRENATAL MORTALITY

Information on pregnancy loss in nonhuman primates is largely provided by studies in the more commonly used Old

TABLE XII

CLINICAL CHEMISTRY REFERENCE VALUES FOR *M. FASCICULARIS*[a]

Parameter	GD 25	(Range)	GD 50	(Range)	GD 75	(Range)	GD 100	(Range)
Cl (m*M*/liter)	110 ± 2	(108–113)	109 ± 2	(105–112)	109 ± 3	(106–114)	109 ± 3	(107–114)
TCO_2 (m*M*/liter)	24 ± 2	(20–27)	24 ± 5	(16–30)	22 ± 5	(12–28)	20 ± 3	(16–24)
K (m*M*/liter)	4.8 ± 0.4	(4.1–5.4)	5.0 ± 0.6	(4.3–5.9)	4.7 ± 0.5	(4.1–5.7)	4.9 ± 0.5	(4.2–5.9)
Na (m*M*/liter)	146 ± 2	(144–148)	146 ± 3	(143–153)	146 ± 3	(143–151)	144 ± 1	(142–147)
Alb (g/dl)	4.0 ± 0.3	(3.7–4.5)	3.5 ± 0.3	(2.8–3.8)	3.1 ± 0.4	(2.4–4.0)	3.2 ± 0.3	(2.7–3.8)
BUN (mg/dl)	21 ± 6	(11–29)	21 ± 6	(15–31)	21 ± 5	(16–32)	18 ± 3	(14–22)
Glucose (mg/dl)	62 ± 9	(46–76)	48 ± 11	(30–64)	46 ± 13	(31–70)	49 ± 6	(40–59)
TP (g/dl)	7.8 ± 0.4	(7.1–8.2)	7.3 ± 0.5	(6.5–7.9)	6.9 ± 0.6	(6.3–8.2)	6.9 ± 0.4	(6.4–7.4)
ALT (U/liter)	47 ± 16	(22–76)	54 ± 34	(15–132)	51 ± 30	(21–112)	50 ± 31	18–117)
ALP (U/liter)	175 ± 49	(114–290)	166 ± 51	(119–275)	123 ± 41	(86–212)	145 ± 31	(104–199)
Ca (mg/dl)	10.0 ± 0.4	(9.5–10.8)	10.0 ± 0.6	(9.2–11.1)	9.7 ± 0.5	(9.0–10.3)	9.2 ± 0.4	(8.8–9.9)
Cr (mg/dl)	0.9 ± 0.1	(0.8–1.0)	0.9 ± 0.1	(0.8–1.1)	0.8 ± 0.1	(0.6–1.0)	0.8 ± 0.1	(0.7–0.9)
P (mg/dl)	3.9 ± 1.1	(1.7–4.7)	3.3 ± 0.8	(1.7–4.5)	2.7 ± 0.8	(1.4–4.3)	3.6 ± 0.6	(2.4–4.3)

Note. Cl, chloride; TCO_2, total carbon dioxide; K, potassium; Na, sodium; AG, anion gap; Alb, albumin; BUN, blood urea nitrogen; TP, total protein; ALT, alanine aminotransferase; ALP, alkaline phosphatase; CA, calcium; Cr, creatinine; P, phosphorus.

[a]Gestational days (GD) 25–100 (N = 10; mean ± SD).

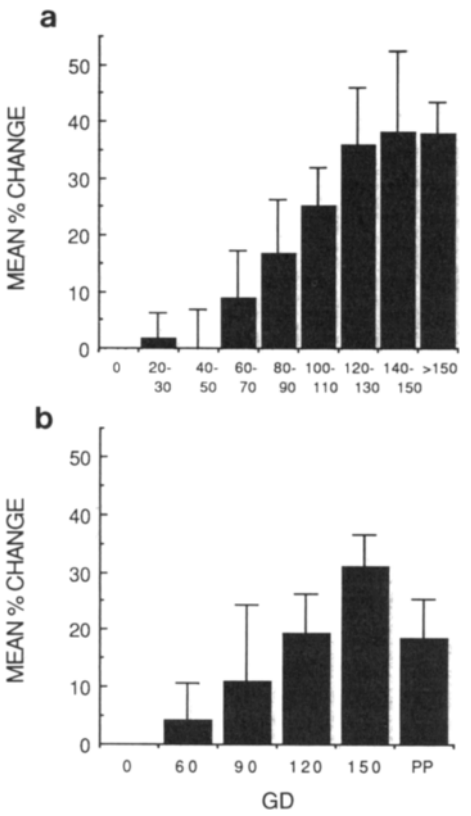

Fig. 5. Maternal body weight (BW) changes during pregnancy for the long-tailed (a) and rhesus (b) macaque (mean ± standard deviation). PP, postpartum; GD, gestational day. Mean percent (%) weight change is calculated in relation to the preconception weight (weight 1) according to:

$$\% \text{ weight change} = \frac{(\text{weight} - \text{weight 1})/}{\text{weight 1}} \times 100.$$

World species, particularly macaques and baboons. Prenatal mortality occurs throughout gestation in these species, but the level is particularly high during the very early embryonic stages when pregnancy confirmation may be uncertain and/or unreliable. Assessment of the magnitude of early embryonic mortality is additionally complicated by the occurrence of "placental sign" (implantation bleeding) which is normally seen in macaques during early pregnancy (see Chapter 9). Morphological examinations of normal and abnormal embryos in the rhesus monkey (Heuser and Streeter, 1941) and baboon (Hendrickx and Binkerd, 1980), as well as the chimpanzee (Heuser, 1940), have provided information on embryonic death during the peri-implantation period. These studies indicate preimplantation losses of 26.3 and 25.0% for rhesus monkeys and baboons, respectively. Corresponding values during the postimplantation period (approximately GD 10–22) have been estimated at 14.3% in baboons, 28% in rhesus monkeys, and 50% in chimpanzees. The increased frequency of prenatal loss during the early stages of pregnancy has also been well-demonstrated in a marmoset breeding colony (Heger *et al.*, 1988). Following a 30% implantation failure rate (includes missed fertilizations and preimplantation losses), 28% of implantations failed prior to organogenesis.

During the subsequent period of organogenesis (approximately days 20–50), the incidence of embryonic loss is significantly lower for several nonhuman primates than in the peri-implantation period. Microscopic examination of embryos of five Old World species (green monkey, long-tailed, rhesus, and bonnet macaques, and baboons) indicate embryonic mortality rates of 2.4 to 18.2% during organogenesis (Hendrickx and Binkerd, 1980). Heger *et al.* (1988) reported a 20% loss during organogenesis in the marmoset.

Reported figures for the incidence of stillbirths among 11 species of indoor-housed primate colonies, including the green monkey, mangabey, baboon, langur, and five species of macaques, range from 5.9 to 20% (Hendrickx and Binkerd, 1980). These rates are markedly higher than the 2.7% figure reported for free-ranging rhesus monkeys (Koford, 1965) and may be attributable to the artificial housing conditions.

The primary factors which have been implicated in prenatal mortality in nonhuman primates are adverse maternal factors (diet, health, infections, and stress) and various uterine conditions which compromise fetal viability (Small, 1982). Studies carried out in rhesus monkeys indicate that normal prenatal growth is maintained on a protein-deficient diet due to the ability of the gravid primate uterus to adapt to such nutritional restriction (Riopelle, 1985). However, severe protein deprivation during pregnancy results in maternal as well as fetal mortality in this species (Kohrs *et al.*, 1976). The effects of experimentally induced diabetes mellitus on fetal metabolism and well-being have also been examined in rhesus monkeys (Mintz *et al.*, 1972). Adminstration of the pancreatic β cell cytotoxin, streptozotocin, before conception and during the first trimester of pregnancy was associated with a 27% mortality rate during the second and third trimesters. The drug effects included fetal hyperinsulinemia, enhanced pancreatic islet cell responsiveness, enlarged placentas, and polyhydramnios.

Both spontaneous and experimentally induced maternal infections are important factors in reproductive failure in a variety of nonhuman primate species (Hendrickx and Binkerd, 1980). The following infections have been implicated as causative agents in abortions or stillbirths: Chagas' disease (*Trypanosoma cruzi*-like) in marmosets; T-strain mycoplasmas in talapoins and patas monkeys; measles virus in rhesus monkeys; rubella virus in long-tailed monkeys and baboons; and mumps virus in rhesus monkeys.

Observations in rhesus monkey colonies indicate that maternal psychological factors may be as important as maternal physiological conditions during pregnancy for fetal viability. The high level of abortion (50 to 70%) in pregnant animals captured in a native environment and shipped to the United States for experimental purposes may be partially attributable to the high degree of stress associated with handling techniques (Myers,

1972). Experiments carried out in rhesus monkeys to study the effect of maternal stress on pregnancy indicate that excitability and discomfort associated with labor and delivery may have deleterious effects on the fetus. Brief episodes of experimentally induced stress in near-term rhesus monkeys cause fetal deterioration in the form of fetal bradycardia and decreased arterial oxygenation (Morishima *et al.*, 1978) or fetal asphyxia and concomitant disturbances in the acid–base balance (Myers, 1975) as a result of impaired uteroplacental circulation. Boot *et al.* (1985) have also demonstrated that housing conditions (i.e., cage size and density) can adversely affect the pregnancy outcome which may be related to stress.

The effects of environmental influences on pregnancy outcome have been studied in several species. For example, the suppression of reproduction in low-ranking or subordinate females has been linked to increased stress in marmosets (Abbott, 1987). A variety of reproductive deficiencies were noted which included poor infant survival to a complete inhibition of ovulation and infertility. Specific hypothalamic factors (i.e., suppression of GnRH) have been suggested as the mechanism responsible for these changes. Although stress appeared to be contributory in New World species under these conditions and in Old World species as described earlier, other environmental impacts such as airline travel and various forms of restraint and immobilization do not appear to result in an altered pregnancy outcome in captive macaques. It has been noted that for pig-tailed, long-tailed, and rhesus macaques, in addition to baboons, jet transport during various periods of gestation does not alter the rate of viable offspring (Sackett, 1981) or increase the rate of spontaneous abortion (A. G. Hendrickx, unpublished observations). Of the 154 pregnant rhesus females (GD 30 to 150) from 1982 to 1990 shipped via air flight to other institutions for experimental purposes, only 0.7% (1/154) produced a nonviable fetus upon arrival; no abortions either during or within 2 weeks of shipment were observed. Other studies have shown that daily capture of gravid pig-tailed macaques during GD 30–130 does not alter gestational length or survival of the offspring (Newell-Morris *et al.*, 1989). Experience at the CPRC indicates that frequent chair restraint and/or hand-catching by experienced animal handlers during all stages of pregnancy in both rhesus and long-tailed macaques does not affect pregnancy maintenance or outcome (Tarantal and Hendrickx, 1988b, 1989a).

Various types of placental insufficiency have also been associated with high rates of fetal mortality, including infections of the placenta (placentitis) (Kaplan, 1979), impaired placental circulation (infarctions and abruptio placenta), and abnormal placental location (placenta previa). The role of ultrasound in the detection of placental abruptions, either subchorionic or retroplacental, is significant (Tarantal and Hendrickx, 1988b). Utilization of this technique, particularly for concealed hemorrhage, in conjunction with emergency hysterotomy can result in major improvements in maternal mortality in addition to the retrieval of viable fetal tissues for experimental purposes.

In a histological study of stillborn fetuses from a variety of nonhuman primates species, a necropsy examination confirmed placentitis in seven of eight cases as the primary cause of fetal demise. The most commonly isolated organisms responsible for ascending genital infections leading to placentitis and subsequent fetal anoxia were Group D streptococci and β-hemolytic, coagulase-positive *Straphylococcus aureus*. Infection of the placenta was accompanied by infiltration of inflammatory cells, edema, necrosis, and hemorrhage which interfered with fetal oxygenation (Andrews, 1974). Gram-positive cocci, especially α-hemolytic *Streptococcus viridans*, were implicated in 11 of 17 cases of abortions and stillbirths in rhesus monkeys (Swindle *et al.*, 1982). Acute placentitis and fetal bronchopneumonia were the most consistent histopathologic findings in these cases.

Although it is difficult to document dystocia in nonhuman primates because of the high incidence of night births, contributing causes may include cephalopelvic disproportion, positional abnormalities, uterine malformation and inertia, uncontrolled hemorrhage, and toxemia (Hendrickx and Giles-Nelson, 1971). Adverse pregnancy outcomes (i.e., stillbirth) have been associated with breech rather than cephalic presentation in term pregnancies of long-tailed (Cho *et al.*, 1985) and pig-tailed (Goodlin and Sackett, 1983) macaques.

Parturition in marmosets can lead to complications, primarily in primiparous births (Hill, 1969; see Section IX). There is no evidence that has linked litter size with parturition difficulties (Poole and Evans, 1982).

IX. PARTURITION

Anatomically, the reproductive tract of the macaque is similar to the human, although differences in cervical and pelvic structures are significant. This is particularly apparent in both *M. mulatta* and *M. fascicularis* who display a serpentine cervix with the presence of colliculi and several branching crypts and clefts (Clark and Corner, 1935; Tarantal *et al.*, 1990) (see Fig. 6). These anatomical differences preclude the routine use of the endocervical canal as a means for accessing the uterus.

Changes in the uterine cervix during pregnancy have been noted via bimanual palpation for both the rhesus (Mahoney and Eisele, 1978) and long-tailed macaque (Mahoney, 1975a,c); softening may occur initially during GD 90–128. Complete softening has been regarded as a fairly reliable indicator of impending parturition, although frequent palpations are required to detect subtle changes. Cervical softening and dilatation cannot be determined based on the macaque cervix as a whole since three distinguishable zones can soften independently during the course of cervical ripening. In addition, the inner and outer os may dilate independently, either one preceding the other. A method for determining "labor readiness" has been established for the rhesus with the use of a scoring system based on the human Bishop Score (Golub *et al.*, 1988). This method utilizes

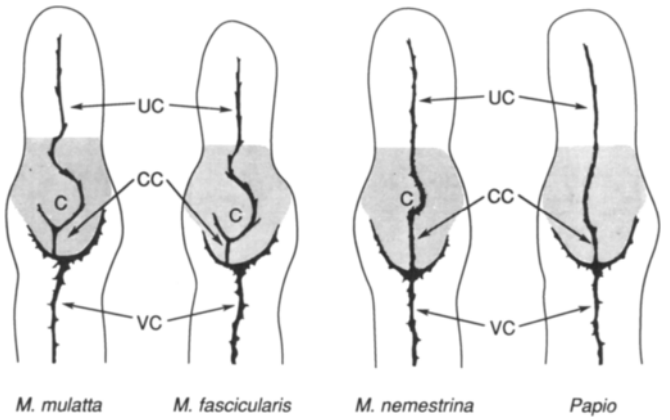

Fig. 6 Comparison of uterine anatomy for four species; shaded area indicates cervix. UC, uterine cavity; C, colliculus; CC, cervical canal; VC, vaginal canal. Note prominent colliculus and blind passages for both the rhesus (*M. mulatta*) and long-tailed (*M. fascicularis*) macaque. A small, less prominent colliculus is present in *M. nemestrina* whereas the baboon (*Papio* spp.) displays a less serpentine cervical canal and no colliculus.

a combination of parameters (cervical position, length, softness, dilatation, fetal head position) and has proven useful for initiating labor induction or to facilitate the prediction of time of birth. Although useful for experimental procedures that require labor induction, from a colony perspective, repeat examinations of this nature are not efficient. Since there is a large variation in the occurrence of parturition in macaques (roughly GD 160–175 for *M. mulatta* and GD 155–170 for *M. fascicularis*), a conservative approach is frequently the best option, provided that information has been collected on the status of the pregnancy and the reproductive history of the dam. This includes a sonographic examination performed during the second trimester which will accurately assess normal growth and development (Tarantal and Hendrickx, 1988b). In addition, potential complications such as a placenta previa can be readily identified, as discussed previously. These observations eliminate questions regarding confirmation of the stage of pregnancy (if not time mated) and the problems that have been encountered when using rectal palpation as the only means for diagnosing pregnancy. In addition, those animals that do not deliver by the anticipated due date can be assessed sonographically in order to determine whether the dam can deliver spontaneously versus performing a cesarean section (A. F. Tarantal, unpublished observations). These factors include fetal presentation (cephalic, breech, transverse), fetal heart rates, length and appearance of the cervix, and fetal colon width/presence of meconium which have all proved useful in the decision-making process. It is well-established that the fetus can alter presentation from breech to cephalic immediately prior to delivery which precludes its use as a reliable indicator of delivery complications. However, depending on the gestational age, status of the dam, and appearance of the cervix, it can be a useful observation, particularly if noted on consecutive examinations in conjunction with bradycardia (<120 bpm) or tachycardia (>200 bpm), and uterine contractions. Regarding the cervix, softening and/or dilatation of both the inner and outer os can be readily identified, and are reliable indicators of the preparturient state (Fig. 7). Delivery can be anticipated within 24 hr when total dilatation and effacement are observed, particularly if the fetal head is engaged. A lack of progression in cervical dilatation in conjunction with advanced gestational age (i.e., GD 175 in the rhesus) and fetal bradycardia or tachycardia obviates the need for a cesarean section. Observations such as agonal gasping (fetal distress) also indicate the necessity for emergency surgery. An additional parameter that has proven useful in interpreting fetal risk is the width of the fetal colon and the volume of meconium. Fetuses with colon widths greater than 9 mm may be at risk for developing a volvulus which, even when delivered quickly, can result in death of the newborn. In general, data collected at the CPRC indicate that a less favorable outcome is observed when delivery has not occurred by GD 175. Only those females who sonographically exhibit complete dilatation of the cervix with a normal fetal heart rate will be returned to their cages at this advanced stage of gestation. Therefore, the decision to deliver by hysterotomy can be enhanced with the incorporation of a variety of sonographic observations in conjunction with maternal physiologic and historical parameters.

Most of the prosimians and New World species deliver during the night; parturition in the prosimians has only been described anecdotally. For most species, there are few indications of imminent parturition. The squirrel monkey will sometimes exhibit a decrease in water consumption, alterations in eating and sleeping habits, and may become withdrawn and inactive. For this species, it is common to house an "aunt" with the dam. Delivery requires from 1 to 2 hr; the mother will assist in the birth process once the head and arms are delivered. Difficulties are frequently encountered due to the weight and size of the newborn, which is roughly 14% of the dam. They are considered potentially viable if weights are a minimum of 84 g (Delort *et al.*, 1976).

Behavioral changes which have been reported to precede parturition in Old World species and great apes include restlessness, altered eating and sleeping habits, frequent urination, intensified grooming, and manipulation of the genitalia (Bo, 1971; Brandt and Mitchell, 1973; Goodlin and Sackett, 1983; Kemps and Timmermans, 1982). Delivery in most species usually occurs nocturnally after a labor of short duration (5 to 7 hr) (Goodlin and Sackett, 1983). Most nonhuman primates assume a squat or upright sitting position during uterine contractions and expulsion of the newborn; this is particularly evident in *Saimiri* (Bowden *et al.*, 1967), *Macaca* spp. (Goodlin and Sackett, 1983; Hartman, 1928; Kemps and Timmermans, 1982), and *Pan* (Nissen and Yerkes, 1943). Hylobates lie on their side during the early stages of labor and assume a sitting position at the time of expulsion; *Pongo* lie on their side or in the supine position just prior to parturition. The gorilla lies prone with the

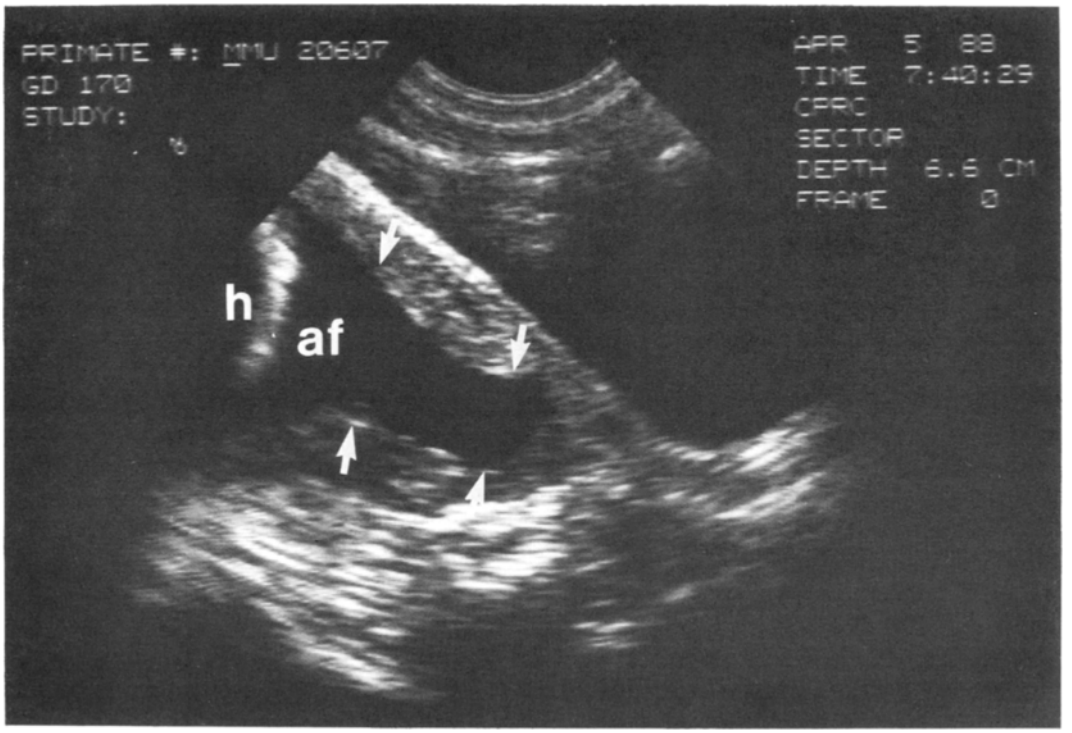

Fig. 7. Sonogram (sagittal section) of a rhesus macaque uterus 24 hr prior to delivery. Note dilated cervix (arrows), amniotic fluid (af), and fetal head (h).

knees parallel to the body and the face may be buried in the palms of the hands. As mentioned earlier, manual aid by the dam during delivery is not uncommon and is described as a characteristic unique to nonhuman primates (Bo, 1971). Vocalization during parturition has been reported for several species, namely *S. sciureus, M. mulatta*, and *P. troglodytes*. For *M. nemestrina*, a pause between delivery of the head and complete emergence of the entire body has been observed in 38 of 40 births with a mean pause duration of 37.2 sec prior to delivery of the body in a single rapid expulsion (Goodlin and Sackett, 1983). A decreasing body temperature of the dam has also been noted prior to delivery (Ruppenthal *et al.*, 1983) which was reported to be more reliable for predicting impending labor than the behavioral signs recorded.

After delivery of the newborn, most primate species will consume the placenta with the exception of the woolly monkey. Cleaning the infant following delivery by licking is a common process, although in some species (i.e., *M. mulatta*) the newborn is temporarily ignored while the mother ingests the placenta (Hartman, 1928). The *S. sciureus* newborn is supported by the mother for only a short time after which it will climb onto the mother's back and begin suckling, usually within 1 hr after birth (Bowden *et al.*, 1967; Clewe, 1969). Studies with marmosets indicate that the newborn depends almost entirely on its own efforts to gain access to the dam which implies that survival during the first hours after birth is totally dependent on vigor. Similar to the marmoset, the tamarin newborn must reach the mother's nipple on its own (Epple and Katz, 1983). Although possibly an artifact of captive conditions, adult male tamarins will retrieve and return newborns which have become separated from their mothers. In contrast, the owl monkey female plays a role in positioning the newborn who will cling in a ventrolateral posture. Infants show a strong upward climbing response and will become very vocal and active if not in this clinging posture. In contrast to many of the other species, they spend relatively little time near the nipple (Dixson, 1983).

X. PERI- AND POSTNATAL ASSESSMENTS

A variety of parameters can be evaluated in the newborn macaque at delivery, either for research-oriented purposes or for establishing normative parameters in vaginally versus surgically delivered infants. The simian Apgar scoring system, which is based on the human Apgar, has been used for assessing both the rhesus (Golub and Gershwin, 1984) and long-tailed macaque (Tarantal and Hendrickx, 1989a) under both conditions. The same general categories of respiratory and heart rates, muscle tone, skin color, alertness, and responsiveness are used in addition to rectal temperature. Ratings are based on a 0–2 point scale for six categories with a maximum score of 12. Scores are obtained at 1, 5, and 10 min of life.

Morphometric evaluation in addition to a complete physical and placental examination are frequently incorporated at delivery. Morphometrics include head measurements (biparietal and occipitofrontal diameters, head circumference), hand and foot lengths, humerus and femur lengths, chest circumference, arm circumference, skinfold thickness (Harpenden skinfold calipers; British Indicators, Ltd.), crown–rump length, and total body weight. Placental examination incorporates total weight (including membranes); recording length, width, and number of discs; branching pattern; evaluation of the umbilical cord (number of vessels, insertion, length); and presence/absence of fibrin, calcification, or infarcts on the maternal and fetal surface. On ocular examination, many long-tailed newborns display bilateral pupillary membranes and unilateral or bilateral hyaloid remnants which may continue for up to 2 weeks of age. Similar observations have been reported for the rhesus (Johnson, 1979).

For those infants assigned to postnatal studies, standarized neurobehavioral examinations have been established for both species (Golub and Gershwin, 1984; Tarantal and Hendrickx, 1989b). The neurobehavioral test battery (NBT) is based on standard human infant examinations (Amiel-Tison *et al.*, 1982; Brazelton, 1984) and is performed on the day of delivery (5 to 6 hr postdelivery if delivered by cesarean section) and again on Days 1–10, 17, and 24 of age. The NBT is designed to evaluate a variety of reflexes and simple behavior patterns, including muscle tone and motor development. The macaque neonate is more advanced in both motor (Castell and Sackett, 1973; Hines, 1942; Taylor *et al.*, 1980) and skeletal development (i.e., ossification) (Watts, 1985) when compared to the human newborn, and develops at a faster rate during the neonatal period (roughly 7 to 10 times greater). The neonatal macaque is capable of crawling and walking within the first 2 to 4 days of life and has a prominent righting and grasping reflex and a high activity level within a short period of time.

Readers are referred to a comprehensive study of reflexes and behavioral characteristics of the squirrel monkey for further information on New World species (Kaack *et al.*, 1979).

XI. LACTATION AND MOTHER–INFANT INTERACTION

The role of lactation in delaying ovulation and subsequent fertility ("postpartum amenorrhea"; see Chapter 9) is an important physiologic event for many nonhuman primate species, particularly Old World monkeys and the great apes. Many, however, such as the New World species, are less affected by this event. In the lesser galago, females whose infants die within 3 weeks of birth have significantly shorter postpartum anovulatory and interbirth intervals when compared to females who raise their infants until weaning (Izard and Simons, 1987). Izard (1987) used milk expression to determine lactation length in three species of galago and found a significantly shorter period for the lesser galago (*G. senegalensis moholi*) when compared to *G. garnettii* and *G. crassicaudatus*. Lactational length of the two latter species did not differ and litter size did not have an effect on lactational length. In the slender loris, it is possible to express milk for up to 153 days postpartum, although clear fluid could be expressed for an additional 11 days (Izard and Rasmussen, 1985). In the slow loris, lactation is approximately equivalent to the length of gestation (6 months); no postpartum estrus occurs in this species, even after death of the young. Unlike the slender loris, the slow loris does not exhibit a hairless lacteal region (Izard *et al.*, 1988).

Lactation in many nonhuman primates results in a higher cortisol level than in noncycling females, and is particularly evident in the squirrel monkey (Coe *et al.*, 1985). Studies have shown that stress-induced lactation can occur when females are exposed to ether anesthesia; the physiological mechanism(s) underlying this response is unclear, although some studies have shown that once milk production is established, it is not dependent on prolactin production. Neither the suckling stimulus nor the high levels of prolactin postpartum will delay the return to ovulation and subsequent fertility in this species (McNeilly *et al.*, 1981). The marmoset infant will nurse for up to 100 days with weaning at roughly 1 month. Infants will take solid food from the hands of their parents or siblings, and can survive entirely on solid food and milk substitutes from $\geq$40 days of age. Plasma prolactin levels of the dams are usually indistinguishable from those of nonlactating females by this time.

Infant owl monkeys develop rapidly and the offspring may transfer from the female to the male on the first day of birth (Dixson, 1983). The offspring first begin to leave the parents between 22 and 46 days and will eat solid food between 35 and 60 days. By the 18th week, they can move independently of their parents and will return if a stressful incident occurs. Little is known regarding lactation in the cebus monkey, but progesterone levels remain low during lactation; weaning begins between 16 and 20 weeks after parturition (Nagle and Denari, 1983). Galagos will usually wean their young naturally at 5 to 6 months of age, at which time the dams will return to normal breeding patterns. Doyle *et al.* (1969) studied maternal behavior of the lesser galago under seminatural conditions and found that during the first 2 weeks of age, attention to the infant is intense with the infants solely dependent on their mothers. At the end of this period, the infants become active and less dependent, will leave the nest box, and take solid food. Between 10 and 11 days, the infants are fully independent and, although they will rest with their mothers, will not suckle. Grooming activity will, however, remain unchanged and will stay at a high frequency throughout the growth process.

Ruffed lemurs tend to remain in the nesting box immediately postpartum; other lemurs in the group will not be permitted access to the newborn until after the first week of age. Infants begin to leave the nest box at roughly 3 weeks of age and by 5 to 7 weeks are experimenting with solid food. Periera *et al.*

(1987) have reported on the care of young ruffed lemurs in forest-like conditions at the Duke Primate Center. Two methods for neonatal care include (1) serial use of multiple ground nests, and (2) "parking" of infants high in the trees. It was suggested that advanced preparation of nest sites, the lack of large predators, alternate maternal and paternal guarding of infants, infant immobility during the absence of the mother, and rapid infant development make these tactics plausible.

A variety of factors other than endocrinologic are known to affect nursing patterns in nonhuman primates. Included are environmental conditions such as positive or negative stimuli, maternal experience, and the mother–infant interaction which contributes significantly to the nursing process. More complex social environments have also been associated with a rapid decline in nursing frequency. Nursing in cage-housed *M. fascicularis* has been monitored in mother–infant dyads by Chance *et al.* (1977). Dams were viewed at 1-min intervals for 8 hr once a week for 3 months. Results indicated that infants nursed for roughly 210 min during the 8 daylight hr at 10 weeks of age, and nursing frequency declined 9.4 min per week until 6 months of age. These findings are similar to nursing patterns of other macaque species (Hinde and Spencer-Booth, 1968; Rosenblum, 1971) and baboons (Rowell *et al.*, 1968). Imitation of one nursing pair by another also appears to have a positive influence. Further data have been collected from long-tailed macaque mother–infant pairs born and reared in the wild then maintained in captivity in family groups (Kemps *et al.*, 1990). In contrast to the rhesus, the long-tailed infant develops a variety of behaviors more quickly during the first 10 days of age, including breaking physical contact with the dam (5–10 days; rhesus from 10 to 20 days). In addition, the dam restricts and rejects the infant to a lesser degree when compared to the rhesus.

The paroxysmal startle is a common response of the macaque infant to rejection and agitation (Chance and Jones, 1974). This characteristic has been noted in both feral and captive colonies, whether raised with the dams or within a nonhuman primate nursery. As noted previously (sonographic BPP), this characteristic has been observed *in utero*, although the reason for the precipitation of this response in the fetus is currently unknown.

XII. REARING OF YOUNG

Rejection of an infant, due to inexperience, aggression, or illness, sometimes necessitates hand or nursery rearing. If artificial rearing is required, infants should be maintained in a heated incubator (30–35°C) with a relative humidity >50%. If the infant is dehydrated, warm fluid may be provided by subcutaneous infusion, stomach tube, or intravenously. Animals can be fed via a dropper, bottle, or syringe pump that can dispense formula one drop at a time. Feedings should be provided on a frequent basis during the first week of life. Monitoring body weight on a daily basis is crucial for adequately evaluating development, although Hamano *et al.* (1990) reported that the frequency of weighing during the neonatal period could be reduced based on individual birth weights. Readers are referred to published information regarding hand rearing and normal neonatal growth rates for both the ring-tailed and ruffed lemurs (Benirschke and Miller, 1981; Meier and Willis, 1984) and the squirrel monkey (Hinkle and Session, 1972).

Nursery rearing of macaques is frequently required when performing postnatal evaluations for experimental purposes. At the CPRC, newborns are housed in infant incubators (either human or hand-fabricated) and receive 5% dextrose orally on the first day of life. After 24 hr, this is replaced with infant formula (Enfamil with Iron, Mead Johnson Nutritional Division) via a small animal nursing bottle. Newborns are fed every 2 hr (7 a.m. to 10 p.m.) until they are able to nurse on their own with the aid of a self-feeder (initially provided on Days 4 to 7 of age). They are provided with formula until roughly 2 weeks of age at which time their diet is supplemented with fruit (banana) and formula-soaked chow (Purina Monkey Chow, 25% protein, Purina Mills, Inc.). At 4 to 5 weeks of age, the infants are adapted to metal quad cages (24 × 18 × 28 inches), at which time their diet consists of formula, soaked and hard chow, and fruit (banana, orange, apple). At approximately 5 to 6 weeks of age, they are further supplemented with Tang liquid drink (Tang, General Foods Corp.) and water via hanging bottles. At 4 to 5 months, formula is discontinued. Once those infants housed singly reach this stage of development, they are paired with another infant of similar age. Within the first week of pairing, surrogates (self-feeders) and bedding are removed from the cage. Only those infants participating in behavioral testing regimens will be housed individually up to this period of time. Colony infants requiring hand rearing (because of health reasons or maternal rejection/aggression) will be paired once adapted to the quad cage.

Daily evaluations of infant status include health, stool consistency, formula intake, and body weight. More complete morphometric evaluations may also performed on a weekly basis, as described under "Peri- and Postnatal Assessments" (see Section X). Daily formula intake and weight gain for both the rhesus and long-tailed macaque during the first 2 weeks of age are shown in Table XIII and Fig. 8, respectively. An initial decline in body weight during the first 4 days of life is typical for both species.

XIII. POSTNATAL GROWTH

Several reports have described postnatal growth patterns for multiple parameters in a variety of species; readers are referred to excellent reviews on this topic by Brizzee and Dunlap (1986) and Watts (1985).

TABLE XIII

FORMULA[a] INTAKE (OUNCES) FOR THE RHESUS (MM; $N = 26$) AND LONG-TAILED (MF; $N = 12$) MACAQUE DURING THE FIRST 2 WEEKS OF AGE

Day[b]	Mm (mean ± SD)	(Range)	Mf (mean ± SD)	(Range)
2	2.8 ± 1.0	(1.2–5.8)	1.4 ± 0.4	(0.9–1.9)
3	3.2 ± 0.9	(1.4–5.1)	1.8 ± 0.4	(1.3–2.6)
4	3.5 ± 0.9	(2.0–6.1)	2.3 ± 0.4	(1.6–3.0)
5	4.4 ± 1.0	(2.5–6.1)	3.1 ± 0.5	(1.9–3.6)
6	5.1 ± 1.4	(2.3–8.0)	3.2 ± 0.6	(1.9–4.0)
7	5.3 ± 1.4	(3.0–8.2)	3.7 ± 0.9	(1.5–5.0)
Total Week 1	24.1 ± 4.6	(13.8–33.0)	15.4 ± 1.7	(11.9–17.1)
8	5.3 ± 1.3	(3.1–7.6)	3.5 ± 1.0	(2.0–4.9)
9	5.3 ± 1.5	(3.1–8.6)	3.7 ± 0.8	(2.1–4.8)
10	5.4 ± 1.3	(3.1–8.0)	3.5 ± 0.8	(2.3–5.1)
11	5.7 ± 1.4	(3.1–8.5)	4.0 ± 0.8	(2.6–5.1)
12	5.3 ± 1.3	(2.8–8.7)	3.9 ± 0.9	(2.6–5.8)
13	5.7 ± 1.7	(3.1–8.7)	4.4 ± 0.7	(3.1–5.6)
14	5.9 ± 1.8	(1.5–9.5)	4.0 ± 1.2	(2.0–5.5)
Total Week 2	38.9 ± 7.6	(26.4–58.5)	27.2 ± 4.8	(19.0–33.8)

[a]Enfamil.

[b]On the day of delivery (Day 1) newborns received 5% dextrose only.

In the squirrel monkey, weight gain proceeds at a steady pace during the first 7 days of life, and by 6 weeks of age the offspring should approximate 150 g (males and females). By 14 weeks, females will weigh roughly 200 g and males 250 g. By 30 weeks, the weights for females and males should be 250 and 400 g, respectively.

A number of growth parameters have been studied in macaques, both at birth and throughout the neonatal, infant, and juvenile periods of development. These include the rhesus (Gavan and Swindler, 1966; Jacobson and Windle, 1960; Kerr *et al.*, 1969; Kirk, 1972; Saxton and Lotz, 1990; van Wagenen and Catchpole, 1956), long-tailed (Shimizu *et al.*, 1988; Tarantal and Hendrickx, 1989a,b; Varavudhi *et al.*, 1989), and pig-tailed macaque (Newell-Morris *et al.*, 1980; Sirianni *et al.*, 1975); the growth of the patas monkey (Sly *et al.*, 1978), cebus (Ausman *et al.*, 1982), baboon (Coelho, 1985; Coelho and Rutenburg, 1989; Glassman *et al.*, 1984; Glassman and Coelho, 1988), and chimpanzee (Brizzee and Dunlap, 1986; Gavan, 1953; Smith *et al.*, 1975) is also well-described. The most relevant studies are those that provide longitudinal data since a more accurate assessment of normal growth and inherent individual variability will be identified utilizing this method. The adolescent growth spurt associated with puberty is not described in this chapter as it is discussed in Section II of Chapter 9.

Birth weights for a variety of species are provided in Table I, and a comparison of growth rates (body weight) for the rhesus and long-tailed macaque is shown in Fig. 9. Weights of rhesus neonates (N=27; 18 males, 9 females) at 4-hr intervals during the first week of life indicate a 10% reduction in body weight (~50 g) by 36 hr which was recovered by the fourth day of age

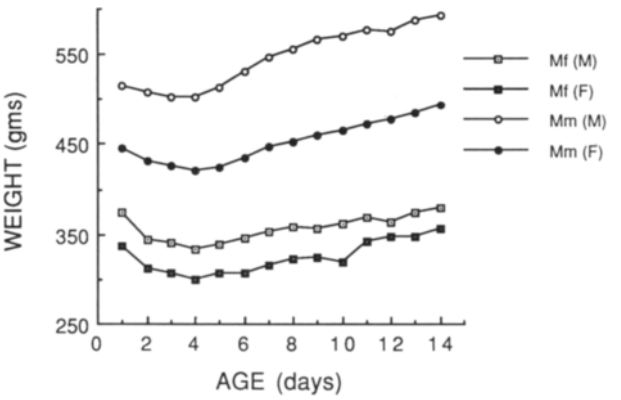

Fig. 8. Mean daily body weights for nursery-reared long-tailed (Mf) and rhesus (Mm) macaques during the first 2 weeks of age. Note decline in body weights during Days 1 to 4. M, male; F, female (mean values shown).

(Kerr *et al.*, 1969). The mean volume intake of liquid diet per kilogram of body weight increased during the first 4 to 6 weeks, then decreased the remainder of the first year. Body weight showed the expected increase during the first year of age, but the rate of weight gain (g/kg/day) reached a peak during the first month then steadily declined thereafter. No significant differences between males and females were shown in this study. Evaluations of weight changes from 2 to 8 years of age in 26 animals (19 male and 7 female) showed a statistically significant difference between the genders, with the male exhibiting a rapid weight gain during the second to fourth year with the greatest growth at 4 years (Kirk, 1972). A comparison of growth rates in the rhesus and chimpanzee indicates that the rhesus grows significantly faster than the chimpanzee, although the rates are equivalent by 1.5 years; no sex differences were observed (Gavan and Swindler, 1966). Differences in growth

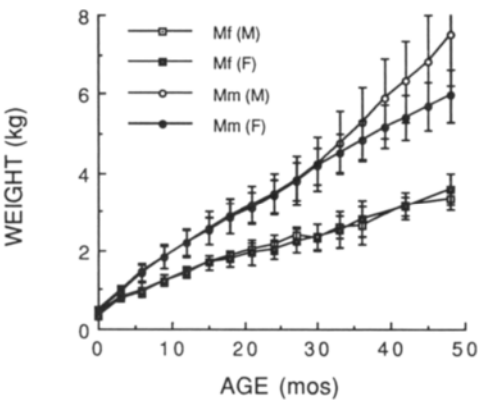

Fig. 9. Postnatal growth curves (body weight) for long-tailed (Mf) and rhesus (Mm) macaques during the first year of age. Long-tailed infants were initially nursery-reared, then pair-housed in adult cages (see Tarantal and Hendrickx, 1989b). Growth data for the rhesus are adapted from van Wagenen and Catchpole (1956). M, male; F, female (mean ± standard deviation).

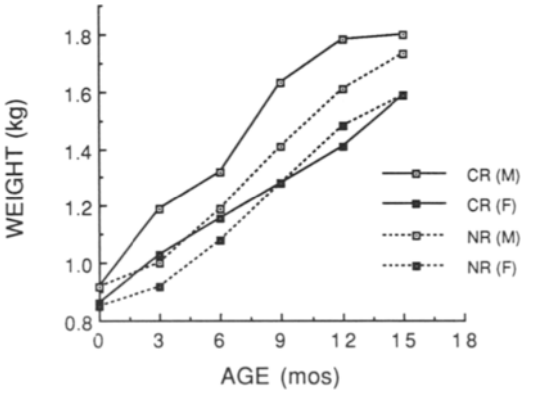

Fig. 10. Comparison of mean body weights for *P. cynocephalus anubis* infants either cage-reared (CR) or nursery-reared (NR). No significant differences for either condition or genders were observed during the first 15 weeks of age. Adapted from Glassman and Coelho (1988). M, male; F, female. From *Am. J. Primatol.*, **16**. Copyright © 1988, John Wiley & Sons, Inc. Reprinted by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

rate patterns between male and female chimpanzees have been noted (Smith *et al.*, 1975); during the juvenile period, the female grows faster than the male whereas late growth proceeds more rapidly in the male.

For *M. fascicularis*, analysis of growth via 15 parameters every 3 weeks during the first 12 weeks of age (N=29) indicated that parameters related to the face and trunk showed a relatively high growth rate when compared to the head, and that the limbs grew at a relatively slow rate (Shimizu *et al.*, 1988). Under natural conditions, growth rates during the first 10 months of age (weaning not achieved until ≥10 months) are very slow (Varavudhi *et al.*, 1989). Changes in body weights under these conditions appear similar to those obtained in captivity (A. F. Tarantal and A. G. Hendrickx, unpublished observations; see Fig. 9).

Several studies evaluating growth and development in the baboon (*Papio cynocephalus anubis*) have been reported. In a study by Glassman *et al.* (1984), gender differences in body weights were noted at 208 weeks of age (N=45 males and 42 females; birth to 7 years). Glassman and Coelho (1988) compared cage- versus nursery-reared infants and found no significant differences for either condition or gender through 15 weeks of age (see Fig. 10). Further studies with this species (Rutenberg and Coelho, 1988) showed that diet had a significant influence on body weight, crown–rump lengths, and triceps girth. In addition, under- or overfeeding effects were long-lasting in females when compared to males (Coelho and Rutenberg, 1989).

Abortion, delivery complications, and a high infant loss are common occurrences for primiparous macaques, particularly those with first pregnancies at a very young age. Data from Small (1982) (Table XIV) indicate that a greater number of stillbirths occurred with 3- and 4-year-old rhesus housed outdoors when compared to older females (≥5 years). It was also noted that the greatest number of first births occurred during the fourth year of age. Later deliveries have also been reported as

TABLE XIV

AGE AT FIRST BIRTH FOR RHESUS FEMALES HOUSED IN OUTDOOR FIELD CAGES AT THE CPRC (1985–1990)[a]

Age (years)	N	% of all ages	Live births		Stillbirths	
			N	% of group	N	% of group
3	118	27.8	89	75.4	29	24.6
4	272	64.0	241	88.6	31	11.4
5	23	5.4	21	91.3	2	8.7
6	7	1.7	7	100.0	0	0

[a]Adapted from Small (1984).

characteristic for 3-year-old primiparous rhesus females when compared to older animals (Wilson *et al.*, 1978). Further reports indicate that primiparous females tend to be more anxious and protective toward their infants and are less likely to bring their offspring to weaning when compared to multiparous females (Kuyk *et al.*, 1977; Seay, 1966). In contrast, however, are data from outdoor-housed *M. radiata* (Small and Rodman, 1981), where first pregnancies did not appear to be at greater risk, and a small group of young *M. fascicularis* (N=15; eight females and six males with one additional experienced adult male) (A. F. Tarantal and A. G. Hendrickx, unpublished observations) group housed in a corn crib. Of the long-tailed females in this cage, the earliest conception recorded was at 2 years 9 months of age, which resulted in a viable male offspring (GD 161). From these eight females, six pregnancies have occurred (2 years 9 months to 4 years 6 months) of which four resulted in viable offspring (GD 150–166; 66.7%), one was a stillbirth (GD 163; 16.7%), and one was pregnant at the time of this writing. Maternal weights at first conception ranged from 2.0 to 3.5 kg. The one female whose pregnancy resulted in a stillbirth conceived a second time 2 months postpartum.

XIV. MALE REPRODUCTIVE PARAMETERS

An extensive review of nonhuman primate male anatomy, semen biochemical characteristics, and sperm morphology has been published by Harrison and Lewis (1986). Similarly, a review on the collection, evaluation, metabolism, and cryopreservation of nonhuman primate semen and spermatozoa has appeared (Wildt, 1986). Readers are also referred to Chapter 9 of this volume for further information.

Artificial Insemination and Electroejaculation

An excellent review of artificial insemination in nonhuman primates has been published by Gould and Martin (1986). Artificial insemination has the potential of supplementing more

conventional methods for providing pregnancies of known age in addition to increasing genetic variability and enhancing production of certain species which, for reasons of numbers or location, are limited. Artificial insemination has been successful in a number of Old World species and great apes, including *M. mulatta, M. fascicularis, P. cynocephalus, P. troglodytes*, and *G. gorilla*. The use of this technique in New World species is limited to *S. sciureus* (Bennett, 1967a,b); no successful reports of artificial insemination in prosimians are available. The overall success rate in all nonhuman primates studied has been low (<50%) compared to other species. One of the factors that contributes to the lack of success in primates is the logistics of obtaining the ejaculate for artificial insemination.

The chimpanzee is the only nonhuman primate species that provides samples following manual masturbation on a predictable basis. Thus, electrostimulation must be used to obtain semen samples in other species; electrodes are placed directly on the penis or in the rectum to provide such stimulation (Gould and Martin, 1986; Hendrickx and Kraemer, 1970; Hendrickx *et al.*, 1978; Kuehl and Dukelow, 1974). Direct sensory stimulation is provided by penile electrodes and precludes the use of anesthesia. Conditioning of the male to ensure a state of relaxation under physical restraint is essential to the success of the procedures; this requirement may also preclude the use of some animals. To perform this procedure, the male is usually placed in a restraint chair and square wave dc current is applied via two metal or nonmetal band electrodes placed around the penis, one near the glans and the other at the base of the shaft (Gould and Martin, 1986; Mastroianni and Manson, 1963; Sarason *et al.*, 1990). The current is applied either in an intermittent surge or is gradually increased over a 10- to 15-sec period to an effective level where it is held for up to 2 min (Settlage and Hendrickx, 1974; Van Pelt and Keyser, 1970). Second or third ejaculates can be collected providing a rest of at least 5 min is provided between stimulations. One of the complications of penile stimulation is trauma, as exhibited by erythema or burning, which can be avoided by the use of a nonmetal or disposable electrical ground place (3M Scotch-plate No. 1149) (Gould and Martin, 1986; Sarason *et al.*, 1990). Successful electroejaculation by the use of rectal probes has been carried out in a number of species and is accomplished by stimulating the male accessory glands to contract and emit semen. This procedure is usually accomplished under light anesthesia. Animals less than 2 kg are restrained in a supine position and larger animals in lateral recumbency or in a restraint chair. The best electrode configuration consists of two or three longitudinal electrodes mounted on the surface of a probe which distends the rectum sufficiently to promote contact of the electrodes with the rectal wall. Electrical current is applied in serial increments with the maximum value raised in 2-V steps at each stage, with six repetitions at each voltage level (Gould and Martin, 1986). The most effective frequency for stimulation is about 20 Hz for larger species and 30–50 Hz for smaller species. Rectal probe electroejaculation is considered safe and only minimal side effects have been reported, e.g., transient urethral blockage due to repeated rectal electroejaculation which has been noted in lemurs (Gould and Martin, 1986).

Significant differences in semen volume occur as a result of the difference in the effector pathway between rectal and penile stimulation. Successful penile stimulation yields ejaculate volumes equal to or exceeding the natural level whereas rectal stimulation yields specimens of variable volumes which are always lower than the natural ejaculate (Gould and Martin, 1986). Penile stimulation is less reliable than rectal stimulation, but, when effective, yields superior results (Hendrickx *et al.*, 1978). Other than semen volume, there was less variability between collection methods for such parameters as current per milliliter, percentage of live sperm, and percentage of motile sperm. Gould and Mann (1988) have shown that, despite a large degree of variability using both methods, sperm counts (count per milliliter and counts per ejaculate) were higher following penile compared to rectal probe ejaculation in rhesus monkeys. No differences were detected in the percentage of live or motile sperm. Additionally, both methods were effective in producing samples approximately 90% of the time, and there was no difference in the fertilizing capacity of the sperm. Since both methods can be used successfully to induce pregnancy, the relative advantage of one method over another may be determined by the species studied, objective of the study, and related factors.

The most important semen parameters are usually considered to be count per milliliter and percentage of progressive motility. The use of a predicted reproductive value (PRV) score based on a weighted evaluation of count per milliliter and progressive motility has been recommended by Harrison (1975). Use of the formula

$$\text{PRV} = \frac{2\,(\text{motility rank value} + \text{concentration rank value})}{3}$$

provides a method for obtaining an equal or better score for a semen sample with a count of 3×10^7/ml and 60% progressive motility compared to one that is 6×10^7/ml with a 30% progressive motility (Gould and Martin, 1986).

Sperm cell counts are done by various methods; a hemocytometer or a variant of the hemocytometer counting chamber has been marketed (Sefi Medical Instruments). Eosin or eosin Y staining is the most routine way of identifying "live" or membrane-intact sperm. Preparation of sperm for artificial insemination should be similar to that used for *in vitro* fertilization (IVF) in that the washing technique should maximize the percentage of progressively motile sperm; remove, by dilution, the antifertility compounds present in the seminal plasma; and provide an adequate energy source. The following method has been successfully employed by Gould and Martin (1986). After collection, semen is allowed to liquify for 30 min at 37°C. The entire coagulum is added to a 6-ml syringe containing the plunger, with the end of the syringe placed in a 15-ml conical tube. After liquefaction occurs, the plunger is removed from the

syringe, allowing the liquified portion of the seminal coagulum to flow into the centrifuge tube for volume measurement. Replacement of the plunger and compression of the remaining coagulum allow extrusion of any remaining fluid and accurate identification of the coagulum volume. A defined medium (e.g., Dulbecco's, BWW, or Ham's F-10) used for subsequent semen washing is generally supplemented with serum. Although recipient serum is usually used which is heated to 56°C prior to use, fetal calf or human cord serum serves as a suitable substitute. The liquified portion of the semen is gently centrifuged at 150 *g* for 5 min at room temperature and the pellet is resuspended in approximately 1 ml of medium. A second centrifugation under the same conditions yields a pellet which is resuspended in 0.2 ml medium and carefully overlaid with another 1.0 ml of medium. The sperm are allowed to swim into the overlying medium for approximately 30 min. An aliquot (0.25–0.5 ml) of the washed sperm, which is evaluated for sperm number, motility, and viability, is then used for artificial insemination.

Since sperm motility is an important indicator of male reproductive function, automated computerized systems have been used to assess movement characteristics such as swimming velocities (Behboodi *et al.*, 1989; Yeung *et al.*, 1989). Improvements in techniques used for image analysis (i.e., from manual to computerized methods) have promoted a higher degree of efficiency (5–9 hr versus 20 min) and also provided additional information such as movement parameters (lateral displacement of the head from the swim path, frequency of the head crossing the swim path).

Although squirrel monkey sperm have been successfully frozen (Denis *et al.*, 1976), there are no confirmed reports of pregnancies resulting after this procedure in New World species. However, cryopreservation of *M. fascicularis* sperm has resulted in a viable offspring after ultrasound-guided intrauterine insemination (IUI) (Tarantal *et al.*, 1990).

The success rate of artificial insemination has varied as a result of the conditions under which the procedure is done; for example, the success rate in several species is higher when intrauterine or intraperitoneal insemination is used compared to intravaginal insemination. With the exception of studies (Czaja *et al.*, 1975; Gould and Martin, 1986) in which conception rates in *M. mulatta* were 52 and 64%, respectively, following IUI, artificial insemination results were similar to those resulting from natural mating. It should, however, be kept in mind that natural conception rates vary from month to month in the most successful colonies. The natural conception rate (number of conceptions divided by number of matings) of both *M. mulatta* and *M. fascicularis* usually varies between 20 and 30%. As stated by Gould and Martin (1986), it is unrealistic to expect that artificial insemination rates would exceed those for natural mating unless an abnormality in sperm transport can be circumvented by sperm placement in a more suitable place to produce a higher fertility rate.

XV. OVULATION INDUCTION

The successful application of artificial insemination depends, to a large degree, on the accurate timing of ovulation (see Section IV). The induction of ovulation by a variety of hormonal regimens can thus enhance the effectiveness of artificial insemination (Gould and Martin, 1986). This methodology is also an essential component in most IVF regimens carried out in both human and nonhuman primates (see the following section). Exogenous gonadotropic hormones from heterologous sources (humans, horses) have been used to stimulate the growth of follicles in one prosimian, the lesser galago (Darney and Franklin, 1982), and in two New World species, the squirrel monkey (Bennett, 1967c; Dukelow and Vengesa, 1986) and the marmoset (Lopata *et al.*, 1988). In the latter species, the prostaglandin $F_{2\alpha}$ analog, cloprostenol, was used to precisely reset the ovarian cycle by inducing premature luteolysis. More extensive use of this technology has been applied to assist in reproductive procedures in Old World monkeys and the great apes. Studies in cynomologus (Balmaceda *et al.*, 1984; Littman and Hodgen, 1985), rhesus (Boatman *et al.*, 1986; Wolf *et al.*, 1989), baboons (Fourie *et al.*, 1987), and chimpanzees have utilized various gonadotropic preparations as well as the antiestrogen, clomiphene citrate, to induce multiple ovulations. One of the major limitations of exogenous ovarian stimulation has been the development of an immune response which prevents repeated animal use in some studies. In this regard, antibodies against pregnant mare serum gonadotropin and human CG have been identified in rhesus monkeys following ovulation induction (Bavister *et al.*, 1986; Ottobre and Stouffer, 1985). In contrast, a FSH–human CG regimen can be frequently repeated in squirrel monkeys with no significant adverse effects on subsequent ovulations (Dukelow and Vengesa, 1986).

XVI. *IN VITRO* FERTILIZATION

There are reports on nonhuman primate *in vitro* fertilization in six species: the marmoset (Lopata *et al.*, 1988), squirrel monkey (Kuehl and Dukelow, 1975), long-tailed monkey (Balmaceda *et al.*, 1984; Ida *et al.*, 1988), rhesus monkey (Boatman *et al.*, 1986; Wolf *et al.*, 1989), baboon (Clayton and Kuehl, 1984; Fourie *et al.*, 1987), and chimpanzee (Gould, 1983). Additionally, normal pregnancies have been reported in marmosets (Summers *et al.*, 1987), baboons (Pope *et al.*, 1986), and rhesus monkeys (Wolf *et al.*, 1989) following cryopreservation of preimplantation embryos obtained following *in vivo* or *in vitro* fertilization. Previously frozen oocytes of the squirrel monkey have also been *in vitro* fertilized (DeMayo *et al.*, 1985). While the use of a nonhuman primate model may further our understanding of gametogenesis, fertilization, and implantation, there are several limitations (e.g., economic restraints, technical dif-

ficulties) to its widespread use in IVF research. Moreover, the use of this technology to produce young may be restricted to extreme cases of infertility or to problem cases of endangered species. Until some of the current problems are resolved, large-scale efforts to increase the production of primate colonies and to preserve species should be directed to the natural techniques of reproduction discussed in this chapter.

XVII. GENETICS

A. Prosimians and New World Species

Among the common prosimians, the galago has a 2*n* chromosome number of 62, the slow loris 50, the mouse lemur 66, the sifaka (*Propithecus coquereli*) 48, the ring-tailed lemur 56, brown lemur 60, the black lemur 44, and the ruffed lemur 46. Extensive studies have been carried out on the chromosomal evolution, particularly of lemurs (Egozcue, 1972; Rumpler and Dutrillaux, 1979). Due in part to the endangered status of prosimians, limited chromosomal research with subsequent selection and breeding has been carried out in these species.

Studies on chromosomes and karyotyping in the New World primates have been extensive due to the discovery in the late 1970s of seven different karyotypes in owl monkeys. The application of this information by breeding animals within the same karyotype has vastly improved production in captivity. The identification of initially seven, and subsequently additional, karyotypes explains some of the earlier reports of conflicting chromosome numbers within this species. Initial studies by Egozcue (1971) reported diploid chromosome numbers of 52 and 56 and Brumback *et al.* (1971) reported polymorphism and diploid numbers of 52, 53, and 54 in a colony of 22 owl monkeys. Ma *et al.* (1976) published a comprehensive evaluation of 330 owl monkeys demonstrating seven distinct karyotypes based on chromosomal numbers distributed among four distinctive phenotypes differentiated by color patterns in the pelage. These various karyotypes displayed diploid chromosome numbers ranging from 46 to 54. In 1978, Ma *et al.* reported two new karyotypes with diploid chromosome numbers of 55 and 56 from Panamanian owl monkeys and, in 1985, an additional two karyotypes with diploid numbers of 47 and 48 were described for Peruvian owl monkeys (Ma *et al.*, 1985). Fifteen gene loci for constitutive enzymes of three karyotypes were confirmed by their presence in seven other karyotypes (Ma, 1984). The continued application of specific karyotypic information to breeding techniques has further increased productivity of this animal in captivity (Elliot *et al.*, 1976).

Squirrel monkeys from different geographical regions of South America with the same diploid number of chromosomes (44) have variable numbers of acrocentric and submetacentric chromosomes, presumably as a result of paracentric inversions (Jones *et al.*, 1973; Jones and Ma, 1975; Ma *et al.*, 1974). Confirmation of these differences and further studies on chromosomal banding patterns in squirrel, capuchin, and owl monkeys were published by Cambefort and Moro (1978). This work has been expanded to study NOR and C-banded polymorphisms in three species and subspecies of squirrel monkeys (Moore *et al.*, 1990). Interestingly, the initial evaluation of the different karyotypes in squirrel monkeys has not led to improvements in breeding; furthermore, the various subspecies will easily interbreed in the wild and captivity. This explains, in part, the lack of research on selection systems for developing genetic lines of squirrel monkeys. Similar to the squirrel monkey, chromosomal evaluation and banding patterns in *C. jacchus, C. penicillata, S. oedipus, S. fusciollis*, and *S. midas* have indicated a chromosomal component of 46 for all these species (Bédard *et al.*, 1978; Pedreira and Peixoto, 1975; Schmid and Glaser, 1977). In contrast, the pygmy marmoset (*Cebuella pygmaea*) has a diploid chromosome number of 44 (Egozcue *et al.*, 1968).

B. Old World Species and the Great Apes

The problem of paracentric inversions described earlier for New World monkeys has not been identified in Old World species; however, one of the main focuses of genetic studies related to reproduction in these species has been the identification of paternity in multi-male breeding groups in order to avoid inbreeding (D. G. Smith, 1986). Genetic markers have been identified for paternity exclusion analysis in *M. mulatta*. By employing this method, the father of a particular offspring can be determined by identifying all sexually mature males which lack a genetic marker which is also absent in the mother but present in the infant. Positive identification of paternity is achieved when all but one of the males in a given group are excluded by at least one polymorphic marker. Males less than 4 years of age are only regarded as possible fathers in instances where all older males are excluded from paternity or when there is evidence of sexual activity during the part of the breeding season when the offspring in question could have been conceived.

Cross-fostering of infants has also been practiced at the CPRC as a practical and effective long-term solution to the problem of inbreeding in large groups of *M. mulatta* (D. G. Smith, 1986). Twenty-three of 32 (72%) male infant swaps to lactating foster mothers within 2 weeks of birth were successful in 1984–1985. This approach offers a simulation of the male emigration that occurs in free-ranging groups and is a step toward preventing genetic subdivision of the breeding groups at the CPRC (S. Smith, 1986). Another consideration for maintaining genetic heterozygosity is the exchange of males between primate facilities not only in the United States but throughout the world.

The consequences of inbreeding in primate populations have been addressed by Packer (1979), Ralls and Ballou (1982), and

D. G. Smith (1986). Packer (1979) reported that the offspring sired by a male *Papio anubis*, who had transferred into a troop containing related females, suffered a higher than normal rate of infant mortality. Ralls and Ballou (1982) reported an increase in infant mortality of inbred young compared to noninbred young in 15 of 16 primate colonies surveyed; the exception was the *M. nemestrina* colony at the Regional Primate Research Field Station, Medical Lake, Washington. Rhesus monkey offspring known to be inbred were matched for age, sex, and cage history with noninbred peers and were compared for mortality, fertility, and morbidity. These factors plus birth weight and infant growth rate have been shown to decline in inbred lines of other species and to influence the survival of rhesus monkey infants (Small and Smith, 1986). The results of this evaluation indicated a lower average birth weight and higher, although not statistically significant, mortality and fertility rates among inbred offspring (D. G. Smith, 1986).

From a management standpoint, culling should be practiced to maintain a balance among founding matrilines and patrilines in each group since genes and combinations of genes that become extinct may not be easily replaced. Culling practices should also ensure that a sufficient number of uniquely identifying genetic markers are carried by each breeding male in the group so that the relative reproductive success of all males can be monitored (D. G. Smith, 1986). It has been observed that males who have poor reproductive success in social groups have above average success in a timed-mated program. Optimal management strategies for breeding heterogeneous members of genetically well-defined nonhuman primate species require a team effort between individuals with interest and training in reproductive biology, veterinary medicine, genetics, primate behavior, demography, and business administration. Application of DNA fingerprinting probes should facilitate management strategies in the future.

The concern with avoiding inbreeding or maintaining genetic heterozygosity in the great apes takes on different proportions than for other species. The outlook for chimpanzees is much better than for other ape species. As mentioned earlier, the exchange of males between colonies is an obvious way of minimizing inbreeding. An alternative to that is the exchange of semen samples between colonies, although the technology of semen preservation must be developed further before this approach becomes feasible (Martin, 1981). Additionally, genetic markers have been developed by the NIH Chimpanzee Breeding and Research Program which will facilitate paternity diagnosis in this great ape (Ferrell *et al.*, 1988).

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CHAPTER 15

Biosafety

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NONHUMAN PRIMATES IN BIOMEDICAL RESEARCH: BIOLOGY AND MANAGEMENT

Biosafety is a most important aspect of laboratory animal medicine, in particular as it relates to nonhuman primates. By definition, safety simply is freedom from danger, injury, or damage; biosafety, freedom from disease or injury caused by living things. This chapter reviews real and potential hazards related to working with nonhuman primates in a biomedical setting and describes measures for keeping people safe from naturally occurring and experimentally introduced pathogenic organisms and from the animals themselves.

PART A. GENERAL BIOSAFETY CONSIDERATIONS

I. INTRODUCTION

A. Historical Perspectives

Nonhuman primates have been recognized as serious threats to human life and health by the research community since the 1930s. Numerous documented cases of human illness and death have been directly and indirectly related to pathogens transmitted from these animals and to other hazards associated with their care and use.

In 1932, a physician died from encephalomyelitis. This was the first of 16 deaths to occur over the next 40 years that had a documented relation to bites inflicted by clinically normal macaques infected with a herpesvirus. After 1973, no cases were seen until the late 1980s, when there were 7 human cases with 2 deaths attributable to *Herpesvirus simiae*. Over the years, hundreds of human cases of tuberculosis have been attributed to contact with nonhuman primates. Scores of human cases of hepatitis A have been related to contact with chimpanzees. Laboratory personnel, many of whom work with nonhuman primates, have an incidence of hepatitis B seven times greater than that in the general population. In 1967, 31 human cases, 7 fatal, resulted from infection with the newly recognized Marburg virus, which was transmitted to workers from African green monkeys.

Literally thousands of people have had varying degrees of subclinical and clinical illness from infection with various zoonotic bacterial, viral, mycotic, parasitic, and other agents transmitted from nonhuman primates. Fortunately, these were less significant and less severe than those just noted. Unfortunately, most of these infections have not been documented in the medical literature.

In recent years, highly fatal epizootics of simian hemorrhagic fever, simian acquired immunodeficiency syndrome, and filovirus infections in nonhuman primate colonies have caused serious concerns to workers, especially during illnesses and die-offs of large numbers of animals. Fortunately, these simian diseases have not been proven to be zoonotic, and no human morbidity or mortality has been associated with these animal outbreaks.

Physical injury to workers inflicted by nonhuman primates has been, and likely still is, quite widespread. Allergies to nonhuman primates have caused occasional problems.

Thousands of workers have had direct contact with numerous species of nonhuman primates, their blood, tissues, and products over recent decades, and thousands more have had direct exposure to nonhuman primate pathogens, both natural and experimental, known and unknown. Yet the recorded incidence of serious injury, morbidity, and mortality is remarkably low in relation to the number of individuals at risk. Indeed, occupational hazards associated with nonhuman primates may be characterized as low risk in nearly all cases but have the potential for grave consequences should certain infections, exposures, or accidents occur.

B. Laws, Regulations, Policies, and Recommendations

The Occupational Safety and Health Administration (OSHA) mandates that employers shall provide and maintain a safe and healthy working environment for employees. Numerous other regulations, laws, policies, recommendations, and guidelines affect nonhuman primate facilities and programs such as the Animal Welfare Act, Public Health Service policy, and "The Guide for the Care and Use of Laboratory Animals" (Pakes, 1985).

Importation and quarantine of nonhuman primates brought into the United States are regulated by the Division of Foreign Quarantine, Centers for Disease Control, Public Health Service. One mission of this division is to safeguard the health of people in the United States, including those in contact with nonhuman primates, by preventing the introduction of etiologic agents from outside the United States. The appearance of filovirus in monkeys at several nonhuman primate importation facilities in 1989–1990 prompted the publication of guidelines for handling nonhuman primates during transit and quarantine [Centers for Disease Control (CDC), 1990a].

Earlier, deaths attributable to *H. simiae* infections of people exposed to macaques in 1987 prompted the publication of guidelines for proper handling to prevent human infection (CDC, 1987b).

C. Responsibilities

Although many aspects of an institution's occupational safety and health program involve the combined responsibilities of numerous parties, the institution per se has the main responsibility of providing a safe working environment for employees and others. Each institution should have one key official, usually the chief executive officer, the director, or a designer, who has the responsibility for administering all aspects of activities involving laboratory animals, particularly nonhuman primates. This responsibility includes the development and implementation of policies and procedures concerned with nonhuman primate care and use. Some institutions, particularly the larger ones that have animal facilities in different locations and/or more than one animal care and use committee, may have an animal policy board that acts in an advisory capacity to the key institutional official.

Animal care and use committees have a major role in ensuring that health and safety requirements are implemented and followed. If institutions have nonhuman primates, it is advisable to have an individual on the animal care and use committee who has expertise in biosafety concerning nonhuman primates. Principal investigators are responsible for understanding and applying established policies and guidelines for animal use

(CDC, 1987d; Richmond, 1991). These investigators should be thoroughly familiar with the biohazards of working with their nonhuman primate models and with potentially hazardous agents, such as microbiologic, toxic, or chemical, that they may be using with nonhuman primates. The researchers also should ensure that they themselves, their laboratory personnel, and others adhere to appropriate practices and procedures when working with nonhuman primates.

Most institutions, particularly the larger ones, have a director of biosafety, and usually the occupational health program is administered through the office of that person. The director of biosafety works closely with institutional officials, boards, committees, and individuals to ensure compliance in this area of responsibility. An institution may have a medical advisory board to formulate policy for working with specific etiologic agents and other issues requiring medical expertise. The occupational health program may be directed by a physician or other health-care personnel who must work closely with institutional officials, employees, and numerous others.

The animal facility director has a major role in institutional biosafety. Usually this individual reviews and approves standard operating procedures and ensures that the facility, programs, and activities conducted within the facility are compliant with institutional and other policies. This director is usually involved in the institutional policy-making decisions affecting the animal facility.

The attending veterinarian, who may also be the animal facility director, is involved in all aspects of health care for the institutional nonhuman primates. This service may be provided on a daily basis, including weekends and emergencies, or less frequently. This individual must be knowledgeable about the natural pathogens transmissible from nonhuman primates to people and of the established routes of transmission. The attending veterinarian must also have close interaction with the institution's physician in attendance. A veterinarian is required to serve on the animal care and use committee, as a voting member, and to review each proposal before its approval. Usually he or she is also consulted by the principal investigator in protocol development. The attending veterinarian must also be knowledgeable about the special attributes of various species of nonhuman primates which pose hazards to workers.

The animal facility manager and supervisory animal care personnel should be involved in writing standard operating procedures. Indeed, their role in implementing and in enforcing standard operating procedures is paramount. Training animal care workers in proper techniques to ensure the safety of the individual is normally their responsibility. Animal care workers have the responsibility of knowing and understanding the standard operating procedures and other institutional safety regulations affecting them and following these mandates explicitly. Some institutions maintain records in which workers sign that they have received training and understand safety procedures/issues. Infractions should be documented for employees found or known to be noncompliant, and they should not be permitted to work with nonhuman primates.

D. Legal Liability

The proposed bloodborne disease standard of OSHA (29CFR Part 1910.1200) is the first federal regulation that specifically addresses the issue of worker exposure to infectious agents in health-care, laboratory, research, production, and other settings. Although this regulation applies to only a relatively limited number of human pathogens (those conventionally transmitted in human blood or body fluids), it is likely that the practices, barrier precautions, and other preventive measures will serve as a generic standard and precedent for activities involving a broad range of infectious agents that may be encountered in biomedical work environments. Further, if animals are subjected to human materials, those animals, their blood, body fluids, and tissues must be handled in compliance with the 1991 OSHA bloodborne pathogen standards.

Negligence—the failure to exercise that degree of care which an ordinary prudent and careful person would exercise under similar circumstances (James, 1985)—is a critical element in determining liability. Industry standards [National Committee for Clinical Laboratory Standards (NCCLS), 1987] and national guidelines (CDC, 1987b, d) form a basis for defining negligence in the context of accepted safety practices for working with infectious materials. Although infection hazards can never be totally eliminated in biomedical work environments, knowledgeable and realistic safety management practices can reduce worker risks and, consequently, liability based on negligence (James, 1985). It is the responsibility of institutional management to develop, implement, and ensure compliance with realistic, prudent, and careful practices appropriate to the assessed risks of the activity being conducted.

II. DISEASE PREVENTION

The ultimate goal of a biosafety program is to prevent, rather than to treat or handle, exposures, infections, and other complications. Disease prevention is optimal control. To achieve this level of control, a basic understanding of the infectious process, risk assessment, animal biosafety levels, and universal and enteric precautions is essential.

A. The Infectious Process

For infections to occur as a result of working with nonhuman primates, three essential elements must be present: (1) an infectious agent or animal that is infected with the agent; (2) a susceptible host or worker who has contact with the animal or agent in the course of experimentation; and (3) a favorable environment. In infectious disease studies, a known pathogen is always present. In other research, the presence of infectious agents is dependent on the disease status of the experimental animals, which may harbor known or unsuspected infectious agents.

Salmonella, Shigella, H. simiae, hepatitis A virus, and tanapox virus are examples of agents documented to have been transmitted to humans from naturally infected nonhuman primates. Use of specific pathogen-free animals can reduce the potential for infection from animals carrying known agents. An adequate quarantine period that allows a veterinarian time to observe the animal, perform diagnostic tests, and give appropriate treatments or vaccinations can help prevent the introduction of disease into a stable colony. The susceptibility of workers is determined by their immune status, which is dependent on their prior disease history, vaccination status, and overall health.

Three additional conditions must be met with work-associated infection to occur: the infectious agent must leave the animal, be transmitted to a host, and enter that host. Understanding these mechanisms provides the basis for selecting appropriate biosafety measures.

1. Mode of Escape

To infect a worker, the infectious disease agent must leave the nonhuman primate. This can happen naturally or artificially. Excretion in urine, saliva, and feces and release through skin lesions are examples of natural escape routes. Invasive research procedures such as venipuncture, biopsy, surgery, and necropsy are obvious artificial avenues of escape. Tissues and body fluids removed from animals may contain disease agents. Vectors present on or having access to infected animals may also facilitate the escape of disease agents.

2. Mode of Transmission

The most frequently documented modes of disease transmission in animal research facilities involve contaminated needles or syringes and direct contact with infected animals. These mechanisms account for 40% of all laboratory-acquired infections resulting from documented accidents (Pike, 1976). Animal bites, scratches, and injury on contaminated work surfaces or cages should be included in this category. However, aerosols, because of their easy dissemination are perhaps the most common—though mostly undocumented—mode of transmission.

Aerosols are small particles of solids or liquids suspended in air. They can remain airborne for an extended time. Particles can become aerosolized by any forceful activity. Nonhuman primates produce aerosols simply by their rapid movements and activity. Most husbandry practices, such as using high-pressure water delivered via a hose to remove animal waste from cages, pans, and floors in animal rooms, produce aerosols, as do many routine laboratory procedures such as centrifugation. Workers may contribute significantly to aerosol production. Vigorous removal of bedding from cages, for example, can increase the concentration of airborne microorganisms in an animal room by a factor of 10 to 100. The smaller the particle, the longer it remains airborne, the more likely it is to move with air currents, and the more likely it is to be inhaled. Inhaled aerosolized particles in the 5-μm range are most likely to be retained in deep pulmonary spaces and therefore establish foci of infection. Infectious aerosols are particles that may contain a single microorganism or clumps of microorganisms that have escaped an experimental animal host or some *in vitro* reservoir (e.g., tissue culture flask or syringe). Infectious aerosols may also consist of microorganisms that are attached to inanimate particles, such as dust from animal bedding. Larger aerosol particles (>5 μm) often settle on surfaces near the source, whereas smaller ones may travel some distance before being deposited onto surfaces or being inhaled. It is this dissemination of contaminants that contributes to secondary contact by people who work with animals. Thus, the worker can become a major vehicle of infection by touching contaminated surfaces and transferring the contaminant to him/herself and other people or surfaces.

3. Route of Exposure

Infectious agents can cause disease by four primary routes of exposure: direct parenteral inoculation, inhalation, contact with mucous membranes or broken skin, and ingestion. The route of exposure for work-associated illness may be the same or distinctly different from that associated with the natural disease process.

The most common mechanisms of exposure to infectious agents associated with experimental animal work are (1) direct inoculation by needles, cuts, or abrasions from contaminated items with sharp edges, and via animal bites; (2) contact of the mucous membranes of the eyes, nose, or mouth by spills of contaminated materials, contaminated hands, or contaminated surfaces; (3) ingestion; and (4) inhalation of aerosols generated by accidents, husbandry practices, and experimental manipulations. It should be emphasized that ingestion is a less likely route of exposure today than it was before pipetting aids eliminated mouth pipetting and laboratory practices prohibited smoking and consumption of food and beverages in work areas.

B. Risk Assessment

Agent-specific risk assessment is a subjective and imprecise process. It is part science, part art, and often best guess. Risk assessment is subject to multiple variables. In addition to the criteria discussed previously (see Section II, A), several other indicators of risk assessment are evident.

1. History of Occupational Infection

Much of the information on occupational infections is anecdotal and incomplete. Rates are lacking. Reported cases, or the absence of reported cases, are nevertheless important indicators of occupational infection risks. The series of surveillance summaries by Pike (1976) document the continuing hazard of such infections as tuberculosis, shigellosis, and hepatitis A and B as

well as a number of other infections caused by less prevalent agents.

In contrast, the absence of any documented cases of occupationally transmitted cases of Creutzfeldt–Jakob disease (CJD) and the fewer than 30 reported cases of occupationally associated human acquired immunodeficiency virus infection strongly support the conclusion that these agents pose low occupational infection risks, despite the grave consequences should infection occur. A similar point can be made with regarding the fewer than 30 cases of B virus (*H. simiae*) reported in the world literature since 1932. The consequences of B virus infection, however, complicate an objective risk assessment of this sporadic, obscure, and poorly defined disease.

Documented infections and disease outcome in laboratory, animal care, and health-care personnel may provide meaningful information on agent and disease-specific occupational risks. The importance of a single case of occupation-associated disease must be cautiously evaluated as an indicator of occupational risk to infectious diseases.

2. Route of Infection

The route of infection of specific agents is perhaps the single most important indicator of occupational infection risk to personnel working in laboratory and animal research facilities. Some agents (*Plasmodium* spp.) may have only a single portal of entry whereas others (e.g., tuberculosis) may have multiple routes by which infection may occur.

Transmission patterns of infectious agents are typically the same in the community as in research facilities. For example, bacterial enteric pathogens typically produce infection following the ingestion of relatively large numbers of viable organisms in both settings. Ordinary laboratory manipulations of clinical specimens and cultures of these agents pose no demonstrated hazard of infection via the respiratory route. Consistent use of common sense, good laboratory practices, and personal protective equipment such as those described and recommended for Biosafety Level 2 can prevent occupational infections from bacterial enteric pathogens and most primary pathogens. Consistent use of the simple practice of hand washing would prevent many of these infections.

Mycobacterium tuberculosis is representative of agents for which Biosafety Level 3 practices, containment equipment, and facilities are recommended. This agent is prevalent in certain communities and is commonly cultured in clinical laboratories. Transmission in the community and laboratory is primarily via inhalation of infectious droplet nuclei, which typically contain one or only a limited number of viable tubercle bacilli. All manipulations of clinical materials, tissues, cultures, or infected animals may generate aerosols of infectious droplet nuclei. Consequently, the good laboratory practices and personal protective equipment recommended for Biosafety Level 2, supplemented by the regular use of primary containment equipment [biological safety cabinets (BSC)] and engineering controls (directional and nonrecirculating ventilation systems), are essential to safely work with this agent.

For some infectious agents, the usual route of infection in the laboratory animal facility may vary considerably from that normally observed in the community. The rickettsiae and arboviruses, typically transmitted by arthropod vectors in the community, are often transmitted in the laboratory by the inhalation of infectious aerosols and accidental parenteral inoculation.

In general, those agents that may be transmitted by multiple routes, especially by inhalation, pose significantly greater occupational infection risks than those with only a single portal of entry.

3. Infective Dose

The infective dose, or more specifically the ID_{50}, is the estimated number of organisms or virus particles required to produce infection in 50% of normal adult humans exposed by a given route. This number may vary widely with the route of infection, the immune status of the exposed person, and the strain of the challenge organism. The hepatitis B virus (HBV), many of the arboviruses, and the rickettsiae have a theoretical ID_{50} of one organism or infective particle when introduced parenterally. Agents causing tuberculosis, coccidioidomycosis, and histoplasmosis have an ID_{50} on the order of 10 or fewer organisms or infective particles when exposure is via the respiratory route. Oral infective doses for bacterial enteric pathogens may vary from 10^1 organisms for *Shigella* to 10^8 organisms for *Vibrio comma*.

4. Virulence

The capability of a microorganism to produce infection and disease in a host may significantly influence the occupational risk assessment. Fresh field isolates should be considered fully virulent within the limits of the agent. Isolates attenuated by passage on artificial media, tissue culture, or laboratory animals may pose lower infection risks than unmodified agents. The virulence of vesicular stomatitis virus (VSV) appears to be readily reduced by passage in tissue culture. Persons working with laboratory-adapted strains, often in very basic facilities and using minimal containment only, rarely show serologic evidence of asymptomatic infection. Manipulations of fresh field isolates of VSV from livestock are typically associated with increased risks of acute febrile flu-like illnesses in workers.

Attenuated strains of yellow fever, vaccinia, and polioviruses are safe and effective vaccines for use in humans. These strains maintain infectivity and elicit an antibody response but characteristically do not produce a generalized disease in healthy human adults. Intentional or accidental passage of these vaccine strains in human or animal hosts may, however, significantly increase the virulence and result in human-to-human or animal-to-animal transmission.

Strains attenuated for one species may, however, retain virulence for other related or nonrelated species. Live rabies vaccines attenuated for dogs may produce clinical disease in cats, foxes, and skunks as well as dogs. In the past century, the only two documented cases of rabies in laboratory workers, one in a research facility and the other in a vaccine production facility, resulted from exposure to an attenuated dog vaccine strain (ERA) and a strain fixed by mouse passage (Street Alabama Dufferin), respectively.

5. Survival in the Environment

The physical environment of laboratories and animal facilities is typically hostile to the growth stages of many primary pathogens. Drying, exposure to ultraviolet light, ambient temperatures, lack of nutrients, and residual chemicals and cleaning products on surfaces reduce the survival of most fastidious pathogens outside of the host or selected growth media.

Coxiella burnetii, M. tuberculosis, many systemic and dermatophytic fungi, hepatitis B virus, and spore-forming bacteria are exceptions; they remain viable on surfaces or as droplet nuclei for extended periods. Occupational infections may occur as a result of inhaling infectious droplet nuclei (tuberculosis, Q fever), contact of intact skin (*Microsporum canis*), or exposure of mucous membranes (hepatitis A virus, leptospirosis) with splashes, sprays, or contaminated fluids.

The risks of infection following exposure to agents in the environment are usually secondary to those associated with more direct manipulations of infectious materials. The capability of an agent to survive in the environment directly influences procedures used for decontaminating work surfaces as well as the containment practices, equipment, and facilities used for laboratory and research animal activities.

6. Activity Conducted

The manner in which procedures are performed may significantly influence the infection risk for personnel. Laboratory manipulations of clinical specimens and cultures of *Legionella pneumophila* are commonly handled on the open bench using good laboratory practices. Such activities have not resulted in reported disease. Animal aerosol challenge studies using concentrated liquid cultures may produce infections in exposed personnel typical of the presumed natural mode of transmission.

The quantity and concentration of materials handled may also be associated with increased infection risks. Large volumes of cultures associated with the production of infectious agents may represent an inherently greater infection hazard than activities typical of isolation and identification of pathogens.

Persons having the most intimate and direct contact with experimentally or naturally infected animals, such as veterinarians, animal caretakers, and researchers, are obviously at greater infection risk than those with indirect or no contact.

7. Other Considerations

A number of other considerations influence the assessment of risk in activities involving infectious agents. The availability and use of safe and efficacious vaccines may significantly reduce individual infection risks (e.g., hepatitis B virus, Venezuelan equine encephalitis virus, and smallpox virus vaccines) and also reduce the level of primary containment required for laboratory and animal studies. However, vaccination must *never* be used to eliminate primary containment or personal protective equipment.

The availability and access to prompt and informed medical staff and the use of specific and effective therapy also reduce individual infection risks following overt exposures.

The consequences of infection are unfortunately often confused with the risk of infection. The grave consequences of infection with rabies, *H. simiae*, Creutzfeldt–Jakob agent, and human immunodeficiency viruses (HIV) are not indicative of infection risks of persons regularly working with these agents. Ordinary laboratory manipulations of each of these agents can be safely conducted using good laboratory practices, barrier precautions (gloves, gowns), and containment equipment (Class II biological safety cabinets) recommended for Biosafety Level 2). In the absence of any demonstrated aerosol infection hazard, no safety advantage is gained by working with these agents using the additional physical containment constraints recommended for Biosafety Level 3. Even with such agents as the Marburg virus, which are currently handled only in maximum containment facilities, i.e., Biosafety Level 4, the documented occupational infections in laboratory and health-care workers have resulted from parenteral or other overt exposures to infected humans or animals, tissues, or fluids, not from exposure to infectious aerosols.

C. Animal Biosafety Levels

Four biosafety levels are commonly referred to in working with nonhuman primates and are designated as Animal Biosafety Levels (ABSL) 1, 2, 3, and 4 and correspond very closely to Biosafety Levels (BSL) 1, 2, 3, and 4 for laboratories. Descriptions regarding standard practices, special practices, containment equipment, and animal facilities are detailed in "Biosafety in Microbiological and Biomedical Laboratories" [Centers for Disease Control/National Institutes of Health (CDC/NIH), 1988]. (This excellent publication also classifies most known human pathogens according to the biosafety level and provides agent summary statements.) These four combinations provide increasing levels of protection to personnel and the environment and are recommended as minimal standards for activities involving infected laboratory animals, potentially and known infected, as well as naturally and experimentally infected.

Animal Biosafety Level 1 involves work with viable microorganisms not known to cause disease in healthy adult humans.

TABLE A.I

RECOMMENDED BIOSAFETY LEVELS FOR ACTIVITIES IN WHICH EXPERIMENTALLY OR NATURALLY INFECTED VERTEBRATE ANIMALS ARE USED

Biosafety level	Practices and techniques	Safety equipment	Facilities
1	Standard animal care and management practices	None	Basic
2	Laboratory coats; decontamination of all infectious wastes and of animal cages prior to washing; limited access; protective gloves; and hazard warning signs as indicated	Partial containment equipment and/or personal protective devices used for activities and manipulations of agents or infected animals that produce aerosols	Basic
3	Level 2 practices plus special laboratory clothing; controlled access	Partial containment equipment and/or personal protective devices used for all activities and manipulations of agents or infected animals	Containment
4	Level 3 practices plus entrance through clothes-change room where street clothing is removed and laboratory clothing is put on; shower on exit; all wastes are decontaminated before removal from the facility	Maximum containment equipment (i.e., Class III biological safety cabinet or partial containment equipment in combination with full-body, air-supplid positive-pressure personnel suit) used for all procedures and activities	Maximum containment

Animal Biosafety Level 4 involves dangerous and exotic agents that pose a high individual risk of life-threatening disease (Table A.I). Thus, most nonhuman primate care and use must be conducted according to recommendations for Animal Biosafety Levels 2 and 3, which we have specified in detail from the 1988 CDC/NIH publication as follows.

Animal Biosafety Level 2 Criteria

1. Standard practices
   a. Doors to animal rooms open inward, are self-closing, and are kept closed when infected animals are present.
   b. Work surfaces are decontaminated after use or spills of viable material.
   c. Eating, drinking, smoking, and storing of food for human use are not permitted in animal rooms.
   d. Personnel wash their hands after handling cultures and animals and before leaving the animal room.
   e. All procedures are carefully performed to minimize the creation of aerosols.
   f. An insect and rodent control program is in effect.
2. Special practices
   a. Cages are decontaminated, preferably by autoclaving, before being cleaned and washed.
   b. Surgical-type masks are worn by all personnel entering animal rooms housing nonhuman primates.
   c. Laboratory coats, gowns, or uniforms are worn while in the animal room. This protective clothing is removed before leaving the animal facility.
   d. The laboratory or animal facility director limits access to the animal room only to personnel who have been advised of the potential hazard and who need to enter the room for program or service purposes when work is in progress. In general, persons who may be at increased risk of acquiring infection or for whom infection might be unusually hazardous are not allowed in the animal room.
   e. The laboratory or animal facility director establishes policies and procedures whereby only persons who have been advised of the potential hazard and who meet any specific requirements (e.g., vaccination) may enter the animal room.
   f. When an infectious agent in use in the animal room requires special entry provisions (e.g., vaccination), a hazard warning sign (incorporating the universal biohazard symbol) is posted on the access door to the animal room. The hazard warning sign identifies the infectious agent, lists the name and telephone number of the animal facility supervisor or other responsible person(s), and indicates the special requirement(s) for entering the animal room.
   g. Special care is taken to avoid contaminating skin with infectious material; gloves should be worn when handling infected animals and when skin contact with infectious materials is unavoidable.
   h. All waste from the animal room is appropriately decontaminated, preferably by autoclaving, before being disposed of. Infected animal carcasses are incinerated after being transported from the animal room in leakproof, covered containers.
   i. Hypodermic needles and syringes are used only for the parenteral injection or aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe–needle units (i.e., the needle is integral to the syringe) are used for the injection or aspiration of infectious fluids. A needle should not be bent, sheared, replaced in the sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before being discarded or reused.

j. If floor drains are provided, the drain taps are always filled with water or a suitable disinfectant.
k. When appropriate, considering the agents handled, baseline serum samples from animal care and other at-risk personnel are collected and stored. Additional serum samples may be collected periodically, depending on the agents handled or on the function of the facility.

3. Containment equipment

Biological safety cabinets, other physical containment devices, and/or personal protection devices (e.g., respirators, face shields) are used when procedures with a high potential for creating aerosols are conducted. These include necropsy of infected animals, harvesting of infected tissues or fluids from animals or eggs, intranasal inoculation of animals, and manipulation of high concentrations or large volumes of infectious materials.

4. Animal facilities
   a. The animal facility is designed and constructed to facilitate cleaning and housekeeping.
   b. A sink for washing hands is available in the room that houses infected animals.
   c. If the animal facility has windows that open, they are fitted with screens.
   d. It is recommended, but not required, that the direction of airflow in the animal facility is inward and that exhaust air is discharged to the outside without being recirculated to other rooms.
   e. An autoclave that can be used for decontaminating infectious laboratory waste is available in the same building that contains the animal facility.

Animal Biosafety Level 3 Criteria

1. Standard practices

   Same as for Animal Biosafety Level 2.
2. Special practices
   a. Cages are autoclaved before bedding is removed and before they are cleaned and washed.
   b. Surgical-type masks or other respiratory protection devices (e.g., respirators) are worn by personnel entering rooms that house animals infected with agents assigned to Biosafety Level 3.
   c. Wraparound or solid-front gowns or uniforms are worn by personnel entering the animal room. Front-button laboratory coats are unsuitable. Protective gowns must remain in the animal room and must be decontaminated before being laundered.
   d. The laboratory director or other responsible person limits access to the animal room only to personnel who have been advised of the potential hazard and who need to enter the room for program or service purposes when infected animals are present. In general, persons who may be at increased risk of acquiring infection or for whom infection might be unusually hazardous are not allowed in the animal room.
   e. The laboratory director or other responsible person establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet any specific requirements (e.g., vaccination) may enter the animal room.
   f. Hazard warning signs (incorporating the universal biohazard warning symbol) are posted on access doors to animal rooms containing animals infected with agents assigned to Biosafety Level 3. The hazard warning sign should identify the agent(s) in use, list the name and telephone number of the animal room supervisor or other responsible person(s), and indicate any special conditions of entry into the animal room (e.g., the need for vaccinations or respirators).
   g. Personnel wear gloves when handling infected animals. Gloves are removed aseptically and autoclaved with other animal room waste before being disposed of or reused.
   h. All wastes from the animal room are autoclaved before being disposed of. All animal carcasses are incinerated. Dead animals are transported from the animal room to the incinerator in leakproof, covered containers.
   i. Hypodermic needles and syringes are used only for gavage or parenteral injection or aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe–needle units (i.e., the needle is integral to the syringe) are used. A needle should not be bent, sheared, replaced in the sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before being discarded or reused. When possible, cannulas should be used instead of sharp needles (e.g., gavage).
   j. If floor drains are provided, the drain traps are always filled with water or a suitable disinfectant.
   k. If vacuum lines are provided, they are protected with high efficiency particulate air (HEPA) filters and liquid disinfectant traps.
   l. Boots, shoe covers, or other protective footwear and disinfectant footbaths are available and used when indicated.
3. Containment equipment
   a. Personal protection clothing and equipment and/or other physical containment devices are used for all procedures and manipulations of infectious materials or infected animals.
   b. The risk of infectious aerosols from infected animals or their bedding can be reduced if animals are housed in partial containment caging systems, such as open cages placed in ventilated enclosures (e.g., laminar-flow cabinets), solid-wall and -bottom cages covered by filter bonnets, or other equivalent primary containment systems.

4. Animal facilities
   a. The animal facility is designed and constructed to facilitate cleaning and housekeeping and is separated from areas that are open to unrestricted personnel traffic within the building. Passage through two sets of doors is the basic requirement for entry into the animal room from access corridors or other contiguous areas. Physical separation of the animal room from access corridors or from other activities may also be provided by a double-doored clothes change room (showers may be included), airlock, or another access facility that requires passage through two sets of doors before entering the animal room.
   b. The interior surfaces of walls, floors, and ceilings are water resistant so that they can be cleaned easily. Penetrations in these surfaces are sealed or are capable of being sealed to facilitate fumigation or space decontamination.
   c. A foot, elbow, or automatically operated sink for hand washing is provided near each animal room exit door.
   d. Windows in the animal room are closed and sealed.
   e. Animal room doors are self-closing and are kept closed when infected animals are present.
   f. An autoclave for decontaminating wastes is available, preferably within the animal room. Materials to be autoclaved outside the animal room are transported in a covered, leakproof container.
   g. An exhaust air ventilation system is provided. This system creates directional airflow that draws air into the animal room through the entry area. The building exhaust can be used for this purpose if the exhaust air is not recirculated to any other area of the building, is discharged to the outside, and is dispersed away from occupied areas and air intakes. Personnel must verify that the direction of the airflow if proper (i.e., into the animal room). The exhaust air from the animal room that does not pass through biological safety cabinets or other primary containment equipment can be discharged to the outside without being filtered or otherwise treated.
   h. The HEPA-filtered exhaust air from Class I or Class II biological safety cabinets or other primary containment devices is discharged directly to the outside or through the exhaust system of the building. Exhaust air from these primary containment devices may be recirculated within the animal room if the cabinet is tested and certified at least every 12 months. If the HEPA-filtered exhaust air from Class I or Class II biological safety cabinets is discharged to the outside through the building exhaust system, it is connected to this system in a manner (e.g., thimble-unit connection) that avoids any interference with the air balance of the cabinets or the building exhaust system.

D. Universal and Enteric Precautions

The CDC established the strategy of universal protection from blood and body fluids to address concerns regarding the transmission of HIV in the health-care setting (CDC, 1987c). These guidelines, referred to as "universal precautions," are based on the concept that all patients should be assumed to be infectious for HIV and other bloodborne pathogens. Since this is the assumption when working with nonhuman primates, these universal precautions should be followed when workers are exposed to nonhuman primate blood, other body fluids (amniotic fluid, pericardial fluid, peritoneal fluid, pleural fluid, synovial fluid, cerebrospinal fluid, semen, and vaginal secretions), and, particularly, any body fluid visibly contaminated with blood.

The modes of transmission of HBV and HIV are similar, and in occupational settings blood is the single most important source of HIV and HBV. Both viruses have been transmitted only by percutaneous inoculation or contact with open wounds, nonintact (e.g., chapped, abraded, weeping, or dermatologic) skin or mucous membranes with blood, blood-contaminated body fluids, or concentrated virus. Since HIV and HBV transmission has not been documented from exposure to other body fluids (feces, nasal secretions, sputum, sweat, tears, urine, and vomitus), the CDC does not apply "universal precautions" to these fluids.

Universal precautions apply to saliva only in the dental setting, where it is likely to be contaminated with blood (CDC, 1985). However, transmission of HBV to gibbons and of non-A, non-B hepatitis (NANBH) to chimpanzees by saliva has been reported (Abe *et al.*, 1987); saliva is the major vehicle for the spread of herpes B virus infection, so universal precautions should be observed for all contact work with all nonhuman primates.

General universal precautions address such topics as training and competence, vaccines and other prophylaxis, appropriate protective clothing, the necessity and value of hand washing, and the proper disposition of sharps. These topics are covered elsewhere in this chapter.

More specific "enteric precautions" have been developed and implemented at some institutions because of possible animal cross-infection or infection of workers by animals harboring virulent infectious agents spread by the fecal–oral route or "hand to mouth." Very small, even invisible amounts of infectious material from feces of certain infected nonhuman primates can cause human infection if ingested. Special procedures over and above these should include the standard universal precautions (primarily for animal research involving hepatitis B, non-A, non-B hepatitis, and AIDS) that have been developed for working with nonhuman primates infected with hepatitis A, waterborne non-A, non-B hepatitis, shigellosis, and other enteric infections.

III. FACILITIES MANAGEMENT

A. Security/Restricted Access

Most naturally occurring and experimental diseases of nonhuman primates posing threats to humans are classified in Ani-

mal Biosafety Levels 2 and 3. For etiologic agents in both of these categories, special practices must be implemented as biosafety precautions. The laboratory director or other responsible person establishes policies and procedures whereby only persons who have been advised of the potential hazards and meet any specific requirements (e.g., for immunization) may enter the animal rooms (CDC/NIH, 1988).

Access to nonhuman primate rooms and areas must be limited to authorized personnel. Facility security fences and other barriers to individuals are useful. Measures that control access through facility gates and doors are also helpful, as are guards, patrols, and the use of video cameras in strategic locations around the facility. Both employee and visitor identification procedures enhance security.

Institutions should have procedures in place for dealing promptly and effectively with unauthorized personnel gaining access to animal facilities. Only institutional security, or possibly other properly trained and authorized individuals, and law enforcement officials should be allowed to become involved in confrontational situations that may lead to altercations and violence. Animal care personnel should promptly report knowledge of unauthorized entry of persons to their supervisor, facility manager, or appropriate official.

B. Showers/Lockers

Shower and locker facilities should be provided at the institution for workers having substantial animal contact. Because of the infectious nature of numerous natural and experimental agents and other factors, and even though protective clothing and devices are worn by workers in close contact with nonhuman primates, showering after contact with nonhuman primates or at the end of the workday is usually recommended.

C. Respiratory Barriers

Face masks are recommended for entry into all animal rooms and for all close contact work with nonhuman primates. Although many nonhuman primate facilities continue to use conventional, disposable surgical masks, form-fitting face masks, only slightly more heavy than surgical masks, approved by the National Institute for Occupational Safety and Health, are now being recommended. The 3M No.9970M HEPA filter mask is ideal. These aerosol and splash barriers are the single most practical and effective means of preventing the airborne transmission of pathogens from nonhuman primates to people.

In addition, a face mask or a full-face respirator also acts as a physical barrier in preventing infection from being acquired orally. It also serves as a disciplinary reminder that nonhuman primates require more special precautions than most other research animals, especially in facilities that have other research animals that do not pose serious biohazards.

D. Protective Clothing

Clothing suitable for use in the animal facility should be supplied by the institution, with the possible exception of undergarments. Such items include head covers, face masks, face shields, goggles, laboratory coats, coveralls, scrub suits, gloves, shoes, shoe covers, boots, and the like. Disposable protective items are suitable and preferred in many situations.

E. Laundry

Institutions should provide laundry services for nondisposable apparel worn by workers in close contact with nonhuman primates. Under no circumstances should potentially contaminated clothing worn around nonhuman primates be taken to or worn to the employee's residence for laundering. Either on-site or commercial laundering services are acceptable in most situations; however, institutional facilities should be used to first decontaminate (usually with bleach) clothing exposed to nonhuman primates.

F. Floor Surfaces

Floors in most facilities housing nonhuman primates have smooth surfaces to enhance sanitation. Since daily sanitation practices usually involve water and most times slippery germicidal/detergent chemicals, these floor surfaces pose a significant hazard for physical injury by slipping or falling. Some institutions report more accidents due to slips and falls on wet slippery floors than for any other one category. Fortunately, these accidents are usually relatively minor, but a significant amount of productive time may be lost during healing and recovery.

Floors should be constructed of skid-proof materials, and workers should wear footgear that provides traction on wet, slippery floors. In addition, workers should be instructed on a periodic basis about the physical hazards of working in an animal facility.

G. Pest Control Programs

Primarily because of the feeding and watering methods of institutions and the unsanitary and wasteful habits of nonhuman primates, vermin are usually a major concern. A continuous program is required to prevent, control, and eliminate pests such as cockroaches, flies, and wild or escaped rodents. Vermin problems exist virtually everywhere there are nonhuman primates in both outdoor and indoor facilities.

Cockroaches have been shown to transmit acanthocephalans among squirrel monkeys. Wild rodents are reservoirs for *Yersinia enterocolitica.* Certain species of insects and mosquitoes are known to transmit or serve as mechanical vectors for various

pathogens. Flies also generally reflect a substandard level of sanitation.

The most effective pest control program prevents the entry of vermin into the facility by screening openings, sealing cracks, and eliminating breeding and refuge sites. Pesticides must be used with due discretion; improper use can induce toxic effects on research animals (Hodgson, 1980) and interfere with experimental procedures. Relatively nontoxic compounds (e.g., amorphous silica gels) should be used to control cockroaches, where possible. "Roach motels" that have adhesive surfaces for entrapment of insects may also be used. Pesticides should be used in animal areas only when necessary and then only after a consultation with investigators whose animals will be exposed to them. Applications of pesticides must be in accordance with federal, state, and local regulations. They should be recorded and must be coordinated with the animal care management staff. An integrated pest management approach is essential.

H. Biohazard Signs/Precautionary Information

When infectious materials or infected animals are present in the laboratory, animal room, or containment module, a hazard warning sign that incorporates the universal biohazard symbol should be posted at all laboratory and animal room access doors. In certain situations, posting the hazard warning sign at corridor entrance doors may be sufficient, provided the entire area is subject to the warning. The hazard warning sign identifies the agent, lists the name and telephone number of the laboratory director or other responsible person(s), and indicates any special requirements for entering the laboratory, such as the need for vaccinations, respirators, and other personal protective measures.

These recommendations hold particularly for nonhuman primates that may be harboring natural agents pathogenic to people in addition to experimental infections.

I. Sanitation

Adequate sanitation is essential in every animal facility, especially those housing nonhuman primates, to prevent the spread of diseases among animals and from animals to people in contact with them and to reduce and control vermin. Daily sanitation practices are required for indoor enclosures and cages housing nonhuman primates. Watering and feeding stations for nonhuman primates housed outside should be sanitized often enough to maintain a constant level of adequate sanitation.

J. Decontamination

The decontamination of animal rooms is usually necessary at varying intervals, e.g., after quarantine, when animals have vacated a room, or after an outbreak of disease. Procedures may vary according to the situation from routine decontamination using a solution of sodium hypochlorite (1:100 dilution of household bleach) or a similar sanitizing agent on all floors, walls, doors, and other surfaces in the animal room to fumigation with an agent such as paraformaldehyde for aerosol sterilization.

K. Waste Handling

Nonhuman primates generate large volumes of waste, primarily uneaten food and excrement. These and other waste products should be removed regularly and frequently, usually daily. Most municipalities allow flushing of these materials into sanitary sewer systems, but this may not be the case for certain agents in Animal Biosafety Levels 3 and 4.

Incinerators located in, or adjacent to, the animal facility are ideal for waste disposal. Incinerators should be in compliance with all federal, state, and local regulations. Transporting waste to off-site incinerators requires special containment and handling precautions. Waste cans, if used, should be metal or plastic, leakproof, and equipped with tight-fitting lids that will not come off or leak if the cans are turned over in transit. Containers of animal tissues, carcasses, and hazardous wastes should be lined with leakproof, disposable liners and should exhibit the biohazard warning sign. If wastes must be stored before removal, the waste storage area should be separated from other storage facilities and be free of flies, cockroaches, rodents, and other vermin.

Hazardous wastes should be rendered safe by sterilization, decontamination, containment, or other appropriate means before they are removed from an animal facility. In some areas, landfill waste disposal is used for nonhazardous waste. Waste handling and disposal must be done in a manner that prevents infection in animals and workers and prevents contamination of the environment.

L. Footbaths

Controversy has existed for years regarding the efficacy of footbaths. Prevailing current opinion supports the use of footbaths in certain situations (e.g., at quarantine room access doors; at access doors of nonhuman primate areas where there is a disease outbreak) wherein certain requirements are met. Footbath solutions must be changed often enough to remain fresh and free of organic matter, usually not less than daily. Footgear surfaces must also be free of organic matter; a brush is usually necessary for use on plantar surfaces of boots. Germicidal agents must be selected on the basis of known or suspected pathogens for which containment is desired. Manufacturers' recommendations should be followed regarding dilution.

M. Heating, Ventilation, and Air-Conditioning

Nonhuman primate rooms and areas, particularly quarantine rooms, must maintain pressures negative to surrounding areas.

Ten to 15 air changes per hour appear to provide adequate ventilation for most animal rooms.

Heating, ventilating, and air-conditioning systems should be designed so that operation can be continued, even at reduced capacity, in the event of failure of the primary system. A means for monitoring the system should be established. The animal-occupied areas should be ventilated separately from areas occupied by people (i.e., offices and laboratories).

IV. EQUIPMENT

A. Primary Enclosures, Cages, and Other Equipment

In the design and construction of nonhuman primate cages and equipment, consideration for the safety of the worker must be a main feature. Enclosures must be escape proof and take into consideration the persistent, creative, destructive, and intellectual capacities of most nonhuman primates. Sometimes two or three padlocks are necessary to ensure security, particularly on chimpanzee enclosures. Cages and equipment must have no sharp edges or points that can injure the skin of a worker. Squeeze-back cages are highly recommended where feasible, and the use of transfer boxes, chutes, tunnels, and squeeze mechanisms for nonhuman primates housed in groups is also highly recommended. Shipping crates, which are usually constructed of wood, nails or staples, metal, and wire, must be handled with extreme care and with protective gloves to prevent sticks, cuts, and splinters that might inoculate a pathogen. The type of construction, particularly interior finishes, should be amenable to cleaning and sanitation.

Cages and equipment used with nonhuman primates must be maintained in satisfactory condition. Most nonhuman primates are quite destructive to cages and everything with which they have direct contact. Institutions must provide repair services that are immediately available. "Quick-fix" repairs to cages by animal care personnel and others with such things as coat hangers, wire, and swine nose rings are highly discouraged. In particular, squeeze mechanisms must be maintained in proper working order to prevent injury to both the animal and the worker.

B. Cage Washers

Cage washers must operate at 180°F to sanitize nonhuman primate cages properly. In many instances, such as when moving cages from quarantine areas to the cage washing area, it is necessary to decontaminate the cages before removing them from the animal room per se. Adequately sanitizing these primary enclosures is extremely important because pathogens may be transmitted from animal to animal and also from nonhuman to human primates. A temperature log should be maintained for each day's cage washer operation, or bacterial monitoring should be conducted often enough to ensure that adequate sanitation levels are achieved.

C. Autoclaves

The availability of autoclaves to sterilize items as large as individual primate cages is recommended, especially at facilities where highly infectious natural and experimental agents are present. Routine maintenance and service, temperature gauge logs and charts, spore strips, and other measures are necessary to assure the proper functioning of autoclaves.

D. Biological Safety Cabinets and Clean Benches

Biological safety cabinets of the Class II category are recommended for laboratories, procedure rooms, and possibly other areas in animal facilities where certain Biosafety Level 2 or 3 containment procedures are necessary. Four subclasses of laminar-flow BSCs comprise Class II [National Sanitation Foundation (NSF), 1983], and appropriate selection should be based on facility and program requirements. Vertical or horizontal open-fronted laminar-flow workstations ("clean benches" or "industrial assembly cabinets") are not appropriate to use for activities involving the use of infectious, toxic, or sensitizing materials under any circumstances.

E. Miscellaneous

Numerous equipment items in an animal facility are often necessarily transported from one animal room to another. These include such items as laboratory carts, animal scales, feed containers, feed scoops, restraint gloves and nets, water hoses, and nozzles. To prevent the transmission of animal pathogens from these items, it is essential that they be properly sanitized or sterilized upon removal from the animal room. This is extremely important with regard to quarantine rooms.

V. PERSONNEL MANAGEMENT

It is the responsibility of the institution to ensure that people caring for or using nonhuman primates are qualified to do so. Even qualified people often have a propensity to function with too much flexibility regarding standard operating procedures, institutional policies, and regulations. Thus, personnel management is one of the most difficult categories in which to achieve satisfaction and compliance regarding biosafety and nonhuman primates.

A. Professional Oversight

Institutions having nonhuman primates must provide professional oversight for these programs. Usually collaborative efforts of biosafety officials and veterinarians or others knowledgeable of nonhuman primate zoonoses and experimental pathogens are necessary to formulate individualized standard operating procedures and occupational health/safety programs that satisfactorily meet the needs of the institution.

B. Standard Operating Procedures

Written standard procedures are essential for the operation of a nonhuman primate facility and program. They should be developed by a professional staff member who is quite familiar with each task as well as with the individuals who actually supervise and perform the procedures. Review and concurrence of a peer or higher level institutional official are also desirable. The need for monitoring and enforcing institutional standard operating procedures to ensure worker compliance cannot be overemphasized. These procedures must be periodically reviewed and updated.

C. Facility Supervision

Supervision of nonhuman primate facilities must be conducted by individuals who are qualified and dedicated. Technical supervisors should be knowledgeable of all standard operating procedures and must ensure compliance by all workers.

D. Personal Hygiene/Protective Clothing

High standards of personal cleanliness by animal care staff are essential in preventing the transmission of pathogenic agents from nonhuman primates to the workers. The institution should provide washing, showering, and locker facilities as well as protective clothing and other supplies for workers to maintain adequate levels of personal hygiene.

Workers should change clothing as often as necessary to maintain personal hygiene. Outer garments worn in animal rooms should not be worn outside the animal facility, especially to eating areas and other general public areas.

Individuals should not be permitted to eat, drink, smoke, or apply cosmetics and lip balm or to manipulate their contact lenses in rooms housing nonhuman primates. Separate areas or rooms should be provided for these purposes.

The simple practice of hand washing is considered by some experts to be the single most important personal hygiene practice. Showering after the completion of contact work with nonhuman primates or at the end of the workday is recommended in most situations.

E. Training

Nonhuman primates, in general, pose a greater risk to people in terms of natural pathogens and physical dangers than most other groups of research animals. Experimental pathogens further multiply the dangers. Therefore, in working with nonhuman primates, it is absolutely essential that tasks are performed correctly and safely. To achieve this end, education of the employee is of utmost importance, training is the ultimate responsibility of the institution. Unfortunately, many entry-level employees at a nonhuman primate facility have limited or no knowledge of or experience with nonhuman primates. Thus, an orientation program for new employees is essential. This should include formal instruction, written materials, and on the job training and work assignment with a senior worker.

An ongoing educational program should be in place to ensure that all persons working in an animal care and use program are fully aware of potential hazards involved in their work, e.g., physical dangers involved with the species in use (such as bites, scratches, and allergies), zoonotic organisms, chemical and radiation hazards, and general safety hazards (such as slippery floors, use of live steam in sanitization procedures and sterilization equipment, and lifting of heavy objects). The education program should also ensure that all persons working with animals know how to recognize, prevent, and render on-site emergency treatment of occupational accidents and exposures. Institutions should provide periodic training programs for employees throughout the year.

F. Substantial Animal Contact

Institutions should define what they mean by "substantial animal contact." This phrase is primarily used to describe the work of those individuals who are to be included in various aspects of occupational safety and health programs. Substantial animal contact is usually based on the number of hours per week or month or a percentage of time one is in direct or close proximity to subject animals. These individuals are then usually categorized according to various immunization needs, blood samplings, tuberculosis testings, biosafety apparel and equipment, and other precautions meeting program requirements.

Most institutions do a good job of identifying personnel with substantial animal contact—those individuals working directly and full time in the animal care and use program. It is those individuals with rare or infrequent contact who pose numerous dilemmas.

G. Occasional Animal Contact

Most institutions do a better job of identifying and including personnel working directly and full time in their animal care and use programs (substantial animal contact) than they do identifying those persons having only occasional animal con-

tact. The list of people who may have authorized entry into nonhuman primate facilities and/or animal rooms and who may have very limited, sometimes unauthorized, or no direct contact with nonhuman primates may include plant operations/maintenance personnel, secretaries/office personnel, janitors, security guards, guest scientists, visitors, spouses, children, students, volunteers, sales representatives, and delivery persons, among others. Obviously, limited or restricted access to nonhuman primate areas is highly recommended, and many individuals who may have building access should not be permitted to enter nonhuman primate rooms or holding areas nor should they be allowed to have direct contact with the animals. Many facilities strictly forbid the entry of children under 15 years of age.

Before authorized entry into a nonhuman primate room or area, such individuals should be counseled by a responsible individual or their supervisor regarding any potential health hazards associated with their animal exposure. In addition, the supervisor is responsible for informing persons about and providing instruction in the proper use of equipment and appropriate safety procedures. Many of these workers and individuals must necessarily be escorted and accompanied at all times by supervisory or other knowledgeable workers inside the primate facility.

H. Emergency Procedures

Most emergency procedures should be covered in institutional standard operating procedures. In particular for those institutions housing macaques, a standard operating procedure for human exposure to *H. simiae* by monkey bites and/or scratches is essential.

I. Occupational Health Programs

Occupational health programs are mandatory for personnel who work with nonhuman primates and for others who have substantial contact with them. A model program is presented as Part C of this chapter.

VI. VETERINARY CARE, ANIMAL HEALTH, AND HUSBANDRY PRACTICES

A. Veterinary Care

Aside from certain physical dangers (bites/assaults), most nonhuman primates that harbor no natural or experimental pathogens pose minimal risks to workers. However, nonhuman primates are found, though rarely, to harbor undetected or even presently unknown zoonotic agents, and thus should always be handled in that light. Indeed, astute veterinary care is an essential part of an animal program that aspires to the ideal of one day working with zoonotic agent-free nonhuman primates. Veterinary services are an institutional requirement that may be provided on a full-time, part-time, or consultative basis. If provided by the latter, participation and visitation by the consulting veterinarian should be regular and frequent. Veterinarians working with nonhuman primates should be experienced in the behavior and husbandry of nonhuman primates as well as medicine and surgery.

B. Quality Control

Quality control is essential in maintaining the good health of nonhuman primates as well as for reducing the risk of workers to pathogens inherent with these animals. Whether by institutional provision, contractual services, or some combination thereof, microbiologic, serologic, and histopathologic services must be readily available.

C. Specific Pathogen-Free Production Colonies

Several rhesus monkey production colonies have been established in the United States with federal assistance to provide a supply of specific pathogen-free macaques for biomedical uses. Such colonies are anticipated to be free of naturally occurring pathogens, including all or most of the following: *H. simiae*, filoviruses, retroviruses, *M. tuberculosis*, shigellae, salmonellae, *Y. enterolitica*, *Campylobacter* organisms, endoparasites, and ectoparasites. Over 1000 breeder animals are now in these programs, and 300–400 offspring should be available for sale annually (M. April, personal communication, 1991).

D. Quarantine

Effective quarantine is essential at all nonhuman primate facilities to minimize the introduction of disease agents into established colonies. Quarantine is also necessary at nonhuman primate import facilities to prevent the entry into the United States of certain exotic viruses pathogenic to humans, which are sometimes harbored by imported nonhuman primates. The entrance of filovirus into the United States in late 1989 and early 1990 reemphasized the need for stringent quarantine measures at facilities that import nonhuman primates.

E. Isolation

Nonhuman primates known or suspected of having a contagious disease should be isolated from healthy animals in the colony. Facilities should have a designated room or isolation area and an isolation plan for holding such animals if the need arises.

F. Necropsy Procedures

Necropsies of nonhuman primates pose significant hazards to workers in animal facilities. These hands-on procedures for close visual observation of organs and tissues possibly harboring and releasing pathogens, both known and unknown, into the environment involve considerable risks. Sharp, contaminated necropsy instruments always pose a threat of accidental autoinoculation to the worker. Aerosols may be created when cavities are opened, tissues are incised, and fluids are moving.

If an experimental or natural pathogen is known to be involved, biosafety precautions recommended or commensurate with the biosafety level for that agent should be the minimal criteria. However, the cause of death of nonhuman primates is often undetermined at necropsy for which the associated biohazards are indeed unknown. The minimal protection for anyone conducting a necropsy on any nonhuman primate should be gloves, face mask, and lab coat or other protective clothing.

Where the necropsies are conducted is most important. Most facilities have a room dedicated for necropsy purposes only. These may include such equipment and features as a downdraft necropsy table, a one-pass air system for the room, a Stryker saw, "surgical" lights, and an array of necropsy instruments and tissue collection necessities. For certain work a biosafety cabinet may be used, especially when special pathogens or small nonhuman primates are involved.

Numerous facilities do not have dedicated space for necropsies, and appropriate measures must be made available when needed. An empty animal room that can be decontaminated afterwards may be a suitable location. Normally, necropsies are not conducted in areas housing animals. However, in rare situations when an epidemic of known or unknown origin is ravaging animals in a particular room, the necropsy may be conducted in that room, especially if it has been determined that the remainder of the survivors will be sacrificed. A few United States facilities have the capability of conducting nonhuman primate necropsies in Biosafety Level 4 facilities. Most necropsies in this country require a minimum of BSL 2 recommendations and some require BSL 3.

Because necropsy of nonhuman primates is such potentially dangerous work, only properly trained and experienced personnel should be allowed to conduct nonhuman primate necropsies. Furthermore, proper instruments and equipment to permit safe performance of these procedures are critical.

Safe containment and proper labeling of blood, tissues, and other items to be removed and transported from the necropsy area to another location are important because accidents en route may unnecessarily expose others to pathogens.

The proper disposal of carcasses and animal remains after necropsy is essential. Usually double bagging in leakproof bags with proper identification is necessary to transport for incineration, landfill, or other disposal means.

After necropsy procedures are completed, the necropsy area should be decontaminated. Minimally, the necropsy table, surfaces near the immediate work area, and the floor of the immediate area should be decontaminated with a suitable disinfecting solution such as a 1:50 dilution of household bleach.

G. Physical and Chemical Restraint

Nonhuman primates must be restrained humanely and properly to prevent injury and undue distress to the animal as well as for the safety of the worker.

In some situations, physical restraint of nonhuman primates is necessary. With proper precautions, such restraints may be used for handling certain species of nonhuman primates, infant and very small nonhuman primates, comatose animals, those undergoing training regimens or in studies precluding the administration of exogenous chemicals, and others. In nearly all cases, extreme caution must be used because one is in direct contact with an alert animal capable of inflicting a scratch or a serious penetrating or crushing bite or of transmitting a natural or experimental pathogen.

Persons handling nonhuman primates and using physical restraint must be knowledgeable and experienced. They should never work alone, especially with a potentially dangerous or nonsedated animal in case emergency assistance is required. Workers must be properly attired with protective devices and clothing.

Nets and leather gloves are used in many facilities for physical restraint. They should be free of defects and repaired or replaced as necessary, as should all equipment used in the physical restraint of nonhuman primates. Sharp canine teeth can penetrate leather gloves.

Squeeze-back cages are necessary for working with most nonhuman primates. A number of technical procedures (inspections, bleedings, samplings, etc.) may be performed safely through the cage structure itself, particularly with nonhuman primates that have been trained for certain presentations.

Pole and collar techniques may be satisfactorily employed for certain conditions. Restraint chairs may be used rarely and with due consideration to the length of restraint and distress to the animal.

Chemical restraint is highly recommended in most situations, especially for macaques and other nonhuman primates weighing more than 2 kg. The most commonly used chemical restraint agent is ketamine hydrochloride. Its induction time and effect are variable in certain individual nonhuman primates, and extra caution should be exercised when hands-on contact is first made. Also, some nonhuman primates recover much faster than others, even when appearing well-sedated the previous moment. Certain nonhuman primates that have received multiple injections of ketamine hydrochloride over an extended period may become refractory to the drug. Leather gloves should be worn when removing an anesthetized nonhuman primate from its cage. The gloves, ketamine hydrochloride, needle, and syringe should be nearby at all times while the animal is out of its primary enclosure.

H. Anesthesia, Surgery, Dentistry, and Technical Procedures

Surgical and dental procedures conducted on nonhuman primates present significant biosafety concerns. A preanesthetic regimen may have a chemical restraint component, and precautions listed in Section VI, G are applicable here. Anesthetic agents should be used with caution. When explosive anesthetic agents are employed, floors must be constructed of conductive materials, and explosion-proof electrical outlets should be located 5 feet off the floor. Anesthetic machines should have scavenging devices for safely exhausting waste gases. The use of volatile, explosive agents, such as ether, is discouraged. When they are used, they must be inside explosion-proof hoods.

Particular care and caution must be exercised when using suture needles and various surgical instruments such as scalpels, scissors, towel clamps, and forceps, which have sharp edges or pointed/serrated tips that may puncture, cut, or abrade (even through surgical gloves) the fingers, hands, or other body parts of the surgeon. The potential transmission of *H. simiae* from macaques to humans via these routes must be minimized and avoided. This hazard remains until potentially contaminated surgical instruments, needles, and scalpels have been adequately sanitized, properly autoclaved, or appropriately disposed of.

The surgeon and others handling tissues of potentially infected nonhuman primates, whether naturally or experimentally infected, may consider wearing two pairs of latex surgical gloves; however, the loss of dexterity must be evaluated, and overconfidence must be avoided because wearing double gloves is not in itself fail-safe.

To lessen the chance of human error, it is advisable for the surgeon or individual performing intricate surgical and dental procedures on nonhuman primates, particularly macaques, to do so only when well-rested and relaxed.

In the performance of dental procedures on nonhuman primates, especially macaques potentially infected with *H. simiae*, extreme caution must be used to avoid punctures, cuts, or abrasions to humans with contaminated instruments. In addition, to minimize the risk of pathogens gaining access to mucous membranes, wearing face masks and face shields or goggles is recommended for close contact work, especially if aerosols are created.

Technical procedures, in particular those involving hypodermic needles and other sharps, must be conducted with the utmost of care to prevent one's exposure to a hazardous agent. Needles should not be recapped but rather discarded in appropriate sharps containers, as for other disposable sharps. Syringes should be of the Luer-Lok type to prevent the detachment of needles during injection and the subsequent spraying of aerosols from solutions of medications, fluids, etc., sometimes after blood has been aspirated into the syringe.

PART B. ZOONOSES, BIOHAZARDS, AND OTHER HEALTH RISKS

I. INTRODUCTION

Zoonoses are infections and diseases shared in nature by humans and other vertebrate animals; more than 150 zoonotic diseases have been recognized and described (Schultz, 1983). Biohazards include not only etiologic agents of zoonotic diseases but also those of experimentally induced infectious diseases that can be transmitted from animals to people in a research setting (Muchmore, 1987). Because of their close phylogenetic relationship, nonhuman primates and people share susceptibility to many species-specific pathogens that do not infect other animals. While this makes them invaluable models for studying human infectious diseases, it also gives them the greatest potential for disease transmission when compared with other laboratory animals. Animals carrying endogenous latent viruses (Heberling and Kalter, 1978; Hsiung, 1970; Many, *et al.*, 1991; Wells *et al.*, 1989) may show no clinical disease and thereby present a hidden hazard to laboratory personnel, as do cell cultures made from their tissues (Hsiung and Swack, 1973). In addition, the risk from bites, scratches, and accidental injury, common to all laboratory animal work, is particularly great with nonhuman primates (Gerone, 1983; Muchmore, 1976).

Articles about emerging zoonoses (Schultz, 1983) and emerging viruses (Morse and Schluederberg, 1990) emphasize the need for vigilance. As yet unknown agents with human disease potential may exist even in stable colony animals; thus, nonhuman primates per se should be regarded as biohazards.

Biosafety Level 2 and Animal Biosafety Level 2 practices, containment equipment, and facilities, as recommended by the Centers for Disease Control (CDC/NIH, 1988), should be observed in the care and use of nonhuman primates. In this section, specific recommendations and emphasis for preventing and controlling the spread of certain etiologic agents are given in addition to those prescribed in the Biosafety Level 2 and 3 standards.

When there is suspicion that one these agents is present, CDC should be contacted immediately for guidance and assistance in diagnosis, establishment of quarantine containment and personnel protective measures, as well as disposition of infected animals and decontamination of facilities.

II. VIRAL DISEASES

Even the most species-specific human viruses are transmissible to one or more nonhuman primate species, so the potential of all viruses found in these animals to be passed to people cannot be ignored. The viruses presented in this section are associated with human infection, to have high potential for transmission to

people working with naturally or experimentally infected animals, or to have grave consequences of infection.

A. Hemorrhagic Fevers

The hemorrhagic fever syndrome characterized by high fever, epistaxis, ecchymoses, bleeding of the gastrointestinal tract and other organs, hypotension, and shock is caused by many viruses. Human infection with a number of these agents has been associated with nonhuman primates.

1. Marburg Virus Infection

The Marburg virus was first discovered in 1967, when it caused death in 7 of 31 human cases in simultaneous outbreaks in Germany and Yugoslavia. This disease is also known as "vervet or green monkey disease" because the entire epidemic was traced to a single shipment of African green monkeys (*Cercopithecus aethiops*) from Uganda to Frankfurt and Belgrade via London, where they were kept with other exotic species in the airport animal-holding area. Although data concerning the epidemiologic interrelationships of this outbreak were incomplete, it is noteworthy that the primary human cases were among those individuals who worked with tissue and blood from the animals or human patients; none were among the animal handlers. Eleven primary cases were related to monkey sacrifice or autopsy, 5 to cleaning contaminated equipment, and 5 to surgical manipulation of the animals. One secondary case, the wife of a primary patient, was thought to have been caused by sexual transmission because the virus was demonstrated in the man's semen (Hull, 1968b). There has been one other incident of Marburg virus infection, with 3 cases reported in Africa (Gear *et al.*, 1975).

Subsequent studies in African green, squirrel (*Saimiri sciureus*), and rhesus (*Macaca mulatta*) monkeys showed that experimentally induced Marburg virus infection was fatal to all. Circulating blood levels were $>10^{10}$ virus particles/ml, and virus was excreted in the saliva, urine, and feces of these animals (Haas and Maass, 1971). These findings, and the fact that other studies showed no evidence of a complement-fixing antibody to the Marburg virus in feral animals (Slenczka *et al.*, 1971), indicate that it is not a simian agent and suggest that direct contact and aerosols are the mechanisms for transmission between animals (Haas and Maass, 1971).

The hemorrhagic syndrome just described is seen in both human and nonhuman primates. Respiratory exposures to infectious aerosols, mucous membrane exposure to infectious droplets, and accidental parenteral inoculation are the primary hazards to laboratory or animal care personnel (CDC/NIH, 1988).

Prevention: Biosafety Level 4 practices, containment equipment, and facilities are recommended for all activities using materials of human or animal origin that may be infected with the Marburg virus. These materials include blood, urine, respiratory and throat secretions, semen, and tissues. The historic "Marburg incident" increased awareness of the zoonotic potential of nonhuman primates and was responsible, in part, for many of the practices that are now standard procedures. Observation and documentation of the various types of exposure that resulted in the transmission of the Marburg virus from animals to research workers demonstrated the need for primary protective barriers, such as gloves and long sleeves.

The epidemiologic evidence for initial infection of the monkeys via transmission from other species of exotic animals is a prime example of the need for strict separation of species.

2. Ebola and Other Filovirus Infections

In 1976, outbreaks of severe hemorrhagic fever occurred in Zaire and in the Sudan. A third outbreak occurred in the Sudan in 1979. A virus, named Ebola after a local river, was isolated from patients in both countries. The Ebola virus is related to the Marburg virus and both were later classified in a new virus family, Filoviridae, with one genus, *Filovirus*.

The initial outbreaks occurred in remote areas, and human infection was primarily the result of contact with infected blood from injections with reused syringes and needles at rural hospitals. Some person-to-person transmission resulted from intimate contact with infected persons.

The Ebola virus produces high mortality (30–60%) in humans and rhesus monkeys by causing fulminating hemorrhagic fever and shock. It has several pathologic features in common with other severe viral hemorrhagic fevers such as Lassa fever, and the rhesus monkey model has been used to study the pathophysiology of its shock and hemorrhage (Fisher-Hoch *et al.*, 1985).

In 1989, infections caused by a filovirus closely related to the Ebola virus were detected in cynomolgus (*Macaca fascicularis*) monkeys imported from the Philippines and held in quarantine facilities in Virginia and Pennsylvania. Transmission among monkeys in quarantine facilities occurred; many of the animals died. Four of five animal handlers who had a high level of daily exposure to infected macaques at one facility were found to have serologic evidence of recent infection with a strain of filovirus isolated from the infected monkeys. None had unexplained febrile illness (CDC, 1990a). These episodes documented the first known instances of Ebola-related filovirus infection in imported nonhuman primates in the United States. There have been three different but antigenically related filoviruses implicated in the episodes in the United States.

The incubation period of naturally occurring diseases in humans and experimental infection in monkeys is typically 5 to 9 days (range of 2–15 days). In humans the disease is rapid in onset and is usually characterized by severe fatigue, headache, high fever, muscle and joint pain, and sore throat. Some patients also have conjunctivitis, jaundice, diarrhea, abdominal pain, and a skin rash. In monkeys, the illness may consist of fever,

depression, coma, and death. On postmortem examination, monkeys may have hemorrhages in the liver and other organs and may have blood and fluid in all body cavities. The gross pathology is very similar to that observed with simian hemorrhagic fever.

Although the serologic evidence suggests that this filovirus can infect humans, it has much lower pathogenicity than do its African counterparts. The high level of transmission to animal handlers in this single facility and the possibility of importation of other virulent viruses underscore the importance of strict adherence of quarantine measures for handling monkeys (CDC, 1990a).

The Centers for Disease Control monitor and regulate quarantine facilities that import nonhuman primates into the United States and have published detailed guidelines for handling nonhuman primates during transit and quarantine (CDC, 1990a).

Prevention: Biosafety Level 4 practices, containment equipment, and facilities are recommended for all activities using materials of human or animal origin that may be infected with the Marburg virus. These include blood, urine, respiratory and throat secretions, semen, and tissues. Respiratory exposure to infectious aerosols, mucous membrane exposure to infectious droplets, and accidental parenteral inoculation are the primary hazards to laboratory or animal care personnel (CDC/NIH, 1988).

3. Simian Hemorrhagic Fever

Although no cases of human disease from the filovirus that causes simian hemorrhagic fever (SHF) have been reported, persons who have had contact with monkeys infected with the simian Ebola virus have demonstrated asymptomatic serum conversion (Many *et al.*, 1991). The potential exists for human infection with any virus that infects nonhuman primates.

This extremely virulent disease causes death by disseminated intravascular coagulation within as few as 3 days from onset of the clinical symptoms. These include epistaxis, ecchymosis, ataxia, anorexia, and lethargy. Clinical laboratory findings include patterns consistent with disseminated intravascular coagulation. Abnormal coagulation factors with fibrin degradation products are the earliest indicators of disease, followed by elevation of the liver enzymes, lactate dehydrogenase, γ-glutamyltransferase, and aspartate aminotransferase, and elevated blood urea nitrogen and creatinine from kidney involvement. Pathognomonic lesions found at necropsy include duodenal necrosis and splenic infarction (D. M. Renquist, personal communication, 1989). Confirmation tests include serology, a rhesus peritoneal macrophage assay, immunochemistry, and antigen capture procedures.

Outbreaks of SHF are not frequent, but eight have been reported with 100% mortality of exposed rhesus and cynomolgus (*M. fascicularis*) macaques. Patas monkeys (*Erythrocebus patas*), baboons (*Papio* spp.), and African green monkeys can be asymptomatic carriers of the virus. Failure to exercise good laboratory practices and to observe strict separation of species was responsible for an outbreak in 1972 in which rhesus monkeys were infected because a bottle of ketamine was contaminated with patas monkey blood (10^{-12} dilution). The disease also spreads by contact with infected animals in a room. During one of several outbreaks in 1989, 400 macaques died or were killed to prevent further spread throughout the facility (Renquist, 1990).

Prevention: Any macaque having epistaxis with ecchymoses and unexplained death should be considered to have SHF, and Biosafety Level 2 procedures for protection of other animals and personnel should be stringently followed with particular attention to access control, strict isolation, and personnel practices until a diagnosis is confirmed.

4. Yellow Fever

The RNA flavivirus that causes yellow fever is endemic to tropical areas of the Americans and Africa, where it is transmitted by *Aedes* spp. mosquitoes. This disease is still a major public health problem in tropical areas where most nonhuman primates originate. The World Health Organization (WHO) (1990b) reported extraordinary activity for yellow fever in 1986–1988, with the largest number of cases since 1948 in Africa and since 1952 in South America.

In urban areas of the Americas where *A. aegypti* mosquitoes have not been eradicated, transmission is from person to monkey to person, and the potential for widespread epidemics persists. A sylvatic or jungle form of yellow fever is maintained as endemic by the monkey–mosquito–monkey cycle in the forest canopies. The vectors are *A. africanus* (Haddow, 1969) in Africa and *A. leucocelaenus* in Central and South America (Pinhiero *et al.*, 1981). Human cases are sporadic and are usually related to epizootics in the monkey population or to forestry operations which bring mosquitoes from the treetops to people on the ground. In African urban–rural transition areas, both monkeys and people serve as intermediate hosts.

Yellow fever virus infection in African monkeys is apparently acquired by young animals, in which the disease is mild and immunity develops, as shown by readily demonstrable antibody titers. In New World monkeys, epizootic disease is characterized by fever, anorexia, vomiting, yellow-to-green urine, albuminuria, and jaundice. Human disease is comparable to that seen in New World monkeys.

Prevention: The severity of disease varies markedly among species, but most nonhuman primates are susceptible to yellow fever. Therefore, animals coming from areas in which the virus is endemic could be infected without showing overt symptoms. For this reason, imported monkeys should have certification that they come from an area free of yellow fever; have been held in double-screened, mosquito-proof enclosure; or have been vaccinated against yellow fever.

All imported nonhuman primates that die within 10 days of arrival should be necropsied with special attention to lesions

that may indicate yellow fever. Necrotic, hemorrhagic, and bile-stained organs are seen grossly, and the characteristic histopathologic finding is massive mid-zonal liver necrosis with eosinophilic intracytoplasmic inclusions known as "Councilman bodies," derived from necrotic hepatocytes (Fox *et al.*, 1984).

Biosafety Level 3 is recommended for yellow fever. Thirty-eight laboratory or laboratory-animal associated cases with eight human deaths have been reported. However, the 17D strain is in the Biosafety Level 2 category, provided that personnel working with this virus have been vaccinated (CDC/NIH, 1988). Isolation of the virus from African green monkeys has been reported (Enviro-Control, 1979), and mosquitoes capable of transmitting the virus are common. Therefore, screening with strict control of flying insects is essential for facilities housing newly imported animals from Africa or South America.

5. Dengue

The four RNA-containing flavivirus serotypes that cause dengue fever are arboviruses transmitted by the bite of *Aedes* spp. mosquitoes. Human and nonhuman primates are the natural hosts. The human disease is usually characterized by a "saddle-back" fever of sudden onset with headache, prostration, "breakbone" muscle and joint pain, and a maculopapular rash that appears when the body temperature rises after an afebrile period. However, the dengue viruses can also produce a hemorrhagic fever syndrome and death.

In the United States dengue has been a public health problem primarily in the south, where *A. aegypti* mosquitoes are found. In southeast Asia, where the incidence of dengue-related hemorrhagic fever has been increasing since 1940, dengue is transmitted by *A. albopictus*. Dengue fever is considered one of the viruses with the greatest potential for "emergence" in the near future (Morse and Schluederberg, 1990) because *A. albopictus*, an aggressive mosquito species, was introduced into the United States in 1985 and has already become established in at least 18 states (Francey *et al.*, 1990).

Prevention: Since monkeys are natural hosts of the dengue virus and the disease is endemic in their native habitats, any newly imported nonhuman primates could be a source of human infection. Animal housing, and quarantine areas in particular, should have mosquito-proof screening and stringent flying insect control measures, which may include ultraviolet light electric insect traps in animal rooms. In the jungle, *Aedes* spp. develop in water-holding holes in trees, but in populated areas they prefer man-made containers. It is hypothesized that *A. albopictus* came into the United States in used tire casings. Therefore, part of an arthropid control program must include maintaining the grounds surrounding animal facilities free of cans, containers, and other refuse that could hold water.

B. Herpesvirus Infections

The herpesviruses are among the most prevalent and important of the endogenous primate viruses because a virus that usually produces asymptomatic carriers of latent infection in the native or reservoir host species may regularly cause severe and often fatal disease in another primate species. This is true to such an extent that the latter have sometimes been called "fatal hosts." Infected individuals in the latent phase may shed the virus at any time, particularly when undergoing stress; thus, the potential for spreading infection to workers or other animals is always present.

1. *Herpesvirus simiae*

H. simiae, also known as herpes B virus, B virus, simian B or monkey B virus, cercopithicid herpesvirus, and, more recently, Cercopithecine herpesvirus 1, was first identified in a polio research scientist who died of a rapidly progressive encephalitic in 1932 following a macaque bite (Sabin and Wright, 1934). Since then, this virus has been found to be enzootic among Old World monkeys of the genus *Macaca*, particularly among rhesus monkeys (*M. mulatta*), with prevalence of infection ranging up to 80% or higher. Thousands of rhesus monkeys have been distributed throughout the world in research facilities, zoos, and, not infrequently, in private homes, and people who work with or care for these animals are often exposed to the herpes B virus through bites, scratches, contaminated needle sticks, and other routes. Although the virus usually causes a minimal or undetectable disease in its natural simian hosts, it may cause a rare but rapidly progressive ascending neuropathy and encephalomyelitis associated with high mortality in humans. There have been at least 31 published cases of human *H. simiae* (Palmer, 1987); of the 24 cases reported in detail, 16 (67%) were fatal. In the 14 years preceding 1987 no human cases of *H. simiae* infection were reported. Then there was a cluster of four human cases in Florida (CDC, 1987a) followed by three additional cases in Michigan in 1989 (CDC, 1989) and one case in Texas in 1991 (Dalgard, 1991).

Primary infection with *H. simiae* in macaques may result in gingivostomatitis with characteristic mucosal lesions, but infection probably occurs frequently without such signs. Thus, the absence of oral lesions is not a justifiable reason to relax biosafety standards when handling individual macaques, although the presence of such lesions mandates the most extreme adherence to handling guidelines. The virus remains latent in the host and may reactivate spontaneously or in times of stress, resulting in shedding of the virus in saliva and/or genital secretions. It has been speculated that *H. simiae* may be sexually transmitted among macaques because less than 25% of monkeys under 3 years of age are antibody positive, whereas 72 to 100% of adult rhesus monkeys are reported to be antibody positive (Palmer, 1987).

H. simiae infection in humans is commonly believed to be transmitted by exposure to contaminated monkey saliva through bites and scratches; however, such exposure has not been consistently documented. Nearly all human cases of herpes B virus infection have occurred in persons exposed to monkeys or mon-

key tissues. One instance of person-to-person transmission has been reported (CDC, 1987a). The wife of an infected animal handler, who applied ointment to the perpetic lesions on her husband and then to a small area of dermatitis on her own hand, became infected (Holmes *et al.*, 1990).

The human disease is characterized by a variety of symptoms which usually occur within 5 days to 1 month after exposure. These include, but are not limited to, any or all of the following: vesicular skin lesions at or near the exposure site; aching; chills and other flu-like symptoms; persistent fever; nausea; lethargy; chest pain and difficult breathing; and neurologic symptoms such as itching or tingling at or near the exposure site, numbness, dizziness, double vision, difficult swallowing, and confusion. If untreated, these early signs and symptoms progress rapidly to coma, respiratory failure, and death.

Prevention of *H. simiae* infection in people is of utmost importance. The ultimate protection may be achieved only by using *H. simiae*-free macaques obtained from breeders and sources known to be free of this zoonotic pathogen. To ensure their negative status, these monkeys must be obtained directly from the supplier, transported, and at all times housed and maintained with no contact with other macaques with unknown or questionable *H. simiae* status. Supplies of monkeys free of *H. simiae* are limited, and purchase prices are commensurate.

Currently, most macaques used in biomedical and behavioral research are known or suspected to harbor *H. simiae*. Guidelines for preventing this infection in monkey handlers were published in 1987 (CDC, 1987b). Most institutions that use macaques have developed their own standard operating procedures for handling these species. The following is a composite listing of biosafety concerns and practices regarding macaques throughout the biomedical community.

a. Macaques should be used only when no suitable alternative animal models are available.
b. When feasible, required macaques should be free of *H. simiae* infection and should be maintained under appropriate conditions to ensure this status.
c. All macaques should be regarded as infected because viral shedding is intermittent and can occur in the absence of visible lesions. The routine screening of macaques for evidence of *H. simiae* infection is not recommended. Even animals previously found to be negative for the virus or antibody might be positive at the time of human exposure.
d. Macaques with oral lesions suggestive of active *H. simiae* infection should be quarantined until the lesions have healed to reduce the risk of viral transmission to workers and other macaques.
e. Protective clothing and devices should be worn at all times when working with macaques. When entering an animal room housing macaques, all personnel should wear the following: cap; mask; work uniform, lab coat, or coveralls; rubber gloves; and rubber boots or disposable booties. When approaching within arm's length of caged monkeys, including infants, the following protective devices should also be worn: face shield; protective smock, lab coat or coveralls with long sleeves; and heavy rubber gloves or leather gloves.
f. All macaques should be anesthetized or chemically restrained with ketamine hydrochloride and/or other suitable agents before being removed from their cages. The only exceptions should be comatose animals and infants less than 6 months of age.
g. Cages and other equipment should be free of sharp edges and corners that may cause scratches or wounds to workers. Cages should be designed and arranged in animal housing areas so that the risk of workers being accidentally grabbed or scratched is minimized.
h. Access to areas where macaques are maintained and used should be limited to workers who are properly trained in procedures to avoid risk of infection. All others who must enter the facility for support or program needs must be accompanied by trained workers.
i. Training of personnel to work with macaques should include special education that teaches the nature of *H. simiae* infection with emphasis on the need to prevent bites, scratches, and other exposure to macaque body fluids, especially saliva, as well as the importance of cleaning wounds immediately and promptly reporting all macaque-related wounds, no matter how slight. Personnel should be informed of the early symptoms of *H. simiae* infection and be reminded that early treatment can prevent progression of the disease and that any lesions or illness suggestive of infection should be reported immediately for essential medical evaluation and follow-up. Animal handlers should also be advised that people who are immunosuppressed by medications or underlying medical conditions may be at higher risk for *H. simiae* infection. The training program should also require a working knowledge of standard operating procedures relevant to protective clothing, devices to help prevent bites and scratches, and proper methods of animal restraint. Furthermore, initial training must be followed by continued observation for lapses in the proper performance of procedures.
j. Potential human exposures to *H. simiae* by bites, scratches, and other parenteral or mucosal exposures to saliva or conjunctival secretions from macaques necessitate immediate identification of the particular animal by number and cage or location, if possible. The suspected animal should be chemically restrained and examined by the attending veterinarian for oral lesions, conjunctivitis, or other clinical signs of *H. simiae* infection. At the discretion of the veterinarian, swabs for virus isolation should be taken from the throat, conjunctiva, and any oral lesions. Sterile cotton swabs (some synthetic swabs inhibit virus replication) may be moistened in Hank's phosphate-buffered saline or similar growth medium containing gentamycin (50 mg/ml). After swabbing with a swirling motion to obtain not just fluid but cells from all areas tested and maintaining a sterile technique, the worker should place the swab tip in a culture tube containing 1 ml of Hank's balanced salt solution. The capped vial should be maintained at 4° C until shipped to a diagnostic laboratory. A 2-ml serum sample from the animal should be stored at 4° or −20° C until needed.
k. The human wound(s) should be immediately and thoroughly cleansed with soap and water and then scrubbed with a surgical brush or sponge. Germicidal cleansing agents such as Dakins solution (1:9 buffered sodium hypochlorite) or a 1:10 dilution of household bleach are also recommended in lieu of soap for this cleansing scrub. Wound sites should be expressed to promote bleeding. Exposures should be immediately reported to supervisory personnel, who should refer the exposed person to a medical consultant without delay. The injured employee should be requested to provide a 5-ml blood sample for storage of serum for future reference or testing, as determined by the physician. The employee should again be reminded of the early signs of *H. simiae* infection and the importance of immediately reporting swelling, pruritus, or formation of vesicles at the wound site.
l. Medical consultation and assistance may be obtained from the Centers for Disease Control, Division of Viral and Rickettsial Diseases, Atlanta, Georgia (404 639-3532 and laboratory assistance is available from the Southwest Foundation for Biomedical Research, San Antonio, Texas (210) 674-1410 ext. 280, or the Virus Reference Laboratory, San Antonio, Texas (210) 614-7350.
m. Preventing and treating *H. simiae* infections in exposed people are problematical. Symptomatic human *H. simiae* infection is so rare that few physicians are likely to know how to diagnose and treat it. Unfortunately,

a delay of only a few days in diagnosis and initiation of treatment may make the difference between full recovery and death. Therefore, copies of the most recent CDC recommendations should be readily available for exposed individuals to take to attending physicians, who may not be fully informed. Acyclovir is the drug of choice; its efficacy in preventing the rapidly progressive neurological disease long-associated with human cases has now been documented in several patients. This justifies more aggressive efforts toward early recognition of infected people. There is no evidence that pooled immune serum globulin is effective in preventing or ameliorating *H. simiae* infection. No hyperimmune *H. simiae* human globulin or vaccine against *H. simiae* is currently available.

2. *Herpesvirus saimiri* and Other Herpesvirus Infections

Although there are no data to indicate that *H. saimiri* can cause fatal human infection, this virus, which does cause fatal sarcoma in other monkeys and nonprimate species (e.g., rabbits), will replicate in human tissue cell cultures and is classified as an oncogenic virus by the National Cancer Institute.

Squirrel monkeys are also asymptomatic antibody-positive reservoir hosts of *Herpesvirus tamarinus*, which causes disease in *Saquinus* spp. and *Aotus* spp., ranging from ulcerative and hemorrhagic lesions to focal liver necrosis (Griesemer and Manning, 1973; Whitney, 1976). Human infection with this virus which produced skin pustules, fever, and nonfatal encephalitis has been reported (T-W-Fiennes, 1967).

Prevention: Only one of these, *H. tamarinus*, has produced human infection (Daniel *et al.*, 1972; T-W-Fiennes, 1967). However, one case from an accidental bite or scratch wound indicates that the potential is there. Biosafety Level 2 with universal precautions, including protective clothing, face masks, and disposable gloves for handling all monkeys, and hand washing after working with them is recommended.

A major lesson to be learned from experience with herpesviruses is the importance of strict separation of species.

3. Epstein–Barr Virus and Cytomegaloviruses

Old World primates are naturally infected with agents closely related to the Epstein–Barr virus (EBV) (Ohno *et al.*, 1978), the etiologic agent of human infectious mononucleosis, which is also related to Burkitt's lymphoma and nasopharyngeal carcinoma (Henle *et al.*, 1979). The human disease is characterized by a sore throat, malaise, fatigue, and abnormal lymphocytes; chimpanzees may have tonsillitis and elevated serum enzyme levels (Finkel *et al.*, 1964). Serologic tests may distinguish among current, recent, and past infection (Kieff *et al.*, 1982) but, like other herpesviruses, EBV is carried for life and may be shed in the saliva at any time (Sixbey *et al.*, 1984). Since the chimpanzee variant cross-reacts with human test antigens, it could be a potential source of infection for handlers (Levy *et al.*, 1971).

Practically every known animal species has its own cytomegalovirus (CMV) infection (Hsiung *et al.*, 1971). In humans, CMV infection is a major cause of fetal morbidity and mortality (Altshuler and McAdams, 1971). It causes renal disease in newborns and a mononucleosis-like illness that may include hepatitis in cases acquired later in life (Weller, 1971). This virus is spread primarily via saliva and urine, and there is strong evidence for an increased infection rate among day-care workers who handle young children (Adler, 1989). Although there are no proven human cases of CMV infection related to chimpanzees, it has been suspected (Muchmore, 1971) and the possibility should be considered.

Prevention: Both EBV and CMV are spread primarily by saliva; CMV is also spread by urine. Therefore, universal precautions employed in a Biosafety Level 2 environment should protect workers.

C. Viral Hepatitis Infections

Five different human hepatitis viruses have now been identified, all of which can be transmitted to one or more species of nonhuman primates. In addition, a virulent marmoset hepatitis virus has been described. Two herpesviruses, EBV (Corey *et al.*, 1975) and cytomegaloviruses, may also cause hepatitis.

The clinical and laboratory findings for viral hepatitis are the same for all of these agents, in varying degrees, with elevated serum enzyme levels of aspartate and alanine aminotrasferases and γ-glutamyltransferase, the most consistent. The serologic markers now available for all but one of the human agents are the most important diagnostic tests.

1. Hepatitis A

The hepatitis A virus (HAV) causes what was formerly known as infectious hepatitis. This agent is a unique picornavirus, currently grouped with the enteroviruses (Purcell *et al.*, 1984).

Serologic tests, which can be quantitated to differentiate between the acute and convalescent phases, are available for anti-HAV IgM, which is positive only during the acute phase, and for anti-HAV IgM, which persists and confers lifelong immunity after infection.

More than 100 human cases of hepatitis A associated with newly imported chimpanzees (*Pan troglodytes*) have been reported (CDC, 1971), and sporadic cases still occur from contact with experimentally infected animals. Naturally occurring HAV has also been reported in newly imported owl monkeys (*Aotus trivirgatus*) (LeDuc *et al.*, 1983; Lemon *et al.*, 1990), in African green monkeys, cynomolgus macaques (Shevtsova *et al.*, 1988), and rhesus monkeys (Lankas and Jensen, 1987).

Prevention: Biosafety Level 2, with special emphasis on hand washing and personal hygiene, is recommended for work involving contact with feces from animals and people known to be or potentially infected with HAV. A safe, effective hepatitis A vaccine, now in clinical trials in the United States, is licensed in Europe and should be available soon. However, prophylactic

intramuscular immune serum globulin (ISG) should be given to all nonimmune people exposed or at risk of exposure. Virus shedding begins 7–11 days postinoculation, before clinical or laboratory evidence of hepatitis, which is seen at 22–33 days (Lemon *et al.*, 1990), so animal contact personnel must be given passive protection before experimental HAV infection studies begin. The recommended dose of ISG is 0.02 ml/kg for people to be at risk for 2 to 3 months, which is more than adequate to cover the viral shedding phase (approximately 21 days) of experimentally infected animals. For prolonged exposure, 0.06 mg/kg of ISG should be given every 5 months. For postexposure prophylaxis, a single dose of 0.02 ml/kg should be given as soon as possible, but within 2 weeks after exposure (CDC, 1991a).

2. Hepatitis B

Hepatitis B, caused by the human hepadnavirus (HBV), reproducibly infects only humans and apes, and chimpanzees are the only suitable animal models (Tabor *et al.*, 1983), although gibbons (*Hylobates* spp.) have been used (Scott *et al.*, 1980). The woodchuck hepatitis virus and the hepadnaviruses of other species, including ground squirrels and Pekin ducks, do not infect people.

Hepatitis B, formerly known as "serum hepatitis," is an important human disease with approximately 200,000 new cases annually (WHO, 1989). Of these, 6–10% become chronic carriers, many of whom will develop hepatocellular carcinoma, and almost 4000 of them will die each year from cirrhosis (Alter, 1982). Hepatitis B has been reported to be the most frequently occurring laboratory-associated infection, with an incidence of up to seven times that seen in the general population in some categories of laboratory workers (Pike, 1976).

Transmission of HBV, found in blood, saliva, semen (Scott *et al.*, 1980), cerebrospinal fluid, and urine (Villarejos *et al.*, 1974), is by parenteral inoculation, droplet exposure of mucous membranes, and contact exposure of broken skin. Since the virus may be stable in dried blood or blood components for several days, the potential for entry through inconspicuous breaks in the skin from environmental surfaces must not be ignored (Lauer *et al.*, 1979).

The hepatitis B status of an individual can be determined by testing for serologic markers.

HBsAg: The hepatitis B surface antigen is present only in acute or chronic infection.

Anti-HBs: The antibody to HBsAg is found in convalescent or immunized individuals. Vaccines produce only anti-HBs because there is no viral replication.

Anti-HBc: The antibody to the hepatitis B core antigen is indicative of virus replication and appears in the acute phase after HBsAg. It persists with HBsAg in chronic carriers and with anti-HBs in convalescent individuals. If anti-HBc is present without HBsAg or anti-HBs, the individual must be considered a carrier.

Prevention: Biosafety Level 2 facilities are adequate for containment of these agents. There are now safe, effective vaccines for HBV and all human health-care and chimpanzee contact workers should be given the immunization series of three injections (at 0, 1, and 6 months). The asymptomatic carrier state exists in chimpanzees and gorillas in the wild (Linnemann *et al.*, 1984), thus the hepatitis B status of all captive chimpanzees should be determined so that carriers can be identified and properly handled.

3. Hepatitis C

After tests for serologic markers of both hepatitis A and B were developed and transfusion blood was routinely tested to remove units with hepatitis B infectivity, it became apparent that approximately 10% of transfused patients still had post-transfusion hepatitis (Alter *et al.*, 1989). Ninety percent of these cases were neither A nor B, so this human disease was called non-A, non-B hepatitis. It was transmitted to chimpanzees (Alter *et al.*, 1978), which have proved to be the only reliable animal model for studying this disease (Tabor *et al.*, 1983; Feinstone *et al.*, 1981). After more than 10 years of research using chimpanzees, in which infection is diagnosed by serum enzyme elevation (ALT, GGT) and the presence of characteristic electron microscopic ultrastructural changes in hepatocytes and liver endothelial cells (Pfeifer *et al.*, 1980), the first serologic test for NANBH was produced from chimpanzee plasma by genetic engineering (Kuo *et al.*, 1989).

The virus, then designated as hepatitis C virus (HCV), is now known to be unique, but related to both flaviviruses and pestiviruses, with the hog cholera virus perhaps the most closely related (Houghton *et al.*, 1991). Conservative scientists advise reserving the diagnosis of hepatitis C infection for those cases of NANBH that are positive for antibodies to HCV (anti-HCV) or are positive for HCV by polymerase chain reaction (PCR).

Prevention: There is no evidence that chimpanzees have naturally occurring NANBH, so only experimentally infected animals pose a risk to handlers. The epidemiology of NANBH is similar to that of hepatitis B, so Biosafety Level 2 facilities and procedures are adequate.

4. Hepatitis D

Delta hepatitis is caused by a defective, incomplete DNA-containing virus (HDV) that requires the presence of a hepadnavirus antigen for replication. Therefore, this agent occurs only in hepadnavirus carriers or individuals infected simultaneously with both viruses. It has been studied in woodchucks with WHV and in chimpanzees with HBV.

Prevention: Biosafety Level 2 is recommended. Hepatitis B carrier chimpanzees and contact personnel must be employed in such a way that there is no risk of contaminating negative animals or contracting experimentally induced HDV.

5. Hepatitis E

In the 1980s, a severe, often fatal, enterically transmitted form of NANBH, designated as ET-NANBH to distinguish it from the parenterally transmitted or post-transfusion PT-NANBH, caused several large outbreaks in India, Nepal, Burma, Pakistan, the Soviet Union, Africa, and Mexico (Velasquez *et al.*, 1990). Now known as the hepatitis E virus (HEV), this agent has been isolated, classified as an enterovirus, and experimentally transmitted to owl monkeys, cynomolgus monkeys, and tamarins (Krawczynski and Bradley, 1989; Ticehurst *et al.*, 1992), but no serologic test is available.

Prevention: Although there are no reported cases of spontaneously occurring HEV infection in monkeys, this is a waterborne disease endemic in countries of origin, especially of New World monkeys, so there is potential for this disease to be brought in by newly imported animals. The epidemiology is similar to that of hepatitis A, so special emphasis on enteric precautions under Biosafety Level 2 conditions is recommended because ISG does not confer protection.

6. Callitrichid Hepatitis

In 1981, 7 golden tamarins (*Leontopithecus rosalia*) died at a zoo in the United States and 12 marmosets and tamarins (family Callitrichidae) died at a zoo in England from an acute, highly fatal, apparently infectious hepatitis of unknown origin (Phillips, 1981; Lucke and Bennett, 1982). Soon thereafter, 12 more golden tamarins and 18 callitrichids representing five different species died in epizootics of the same disease in zoos or animal parks in the United States. The sporadic occurrence of epizootics in settings where animals are usually displayed in small family groups suggested that a reservoir species was involved in maintaining and transmitting the virus.

Clinical signs and symptoms of callithrichid hepatitis (CH) are nonspecific and include dyspnea, anorexia, weakness, lethargy, and, frequently, prostration and death. Postmortem findings include jaundice, nonsanguinous pleural and pericardial effusions, subcutaneous and intramuscular hemorrhages, and hepatospenomegaly. Microscopic liver lesions include hepatocellular swelling and necrosis with intracellular acidophilic inclusion bodies (Councilman-like) and mild inflammatory cell infiltration.

The virus isolated from these cases has been characterized as an ultrastructurally typical arenavirus, antigenically related to the Old World arenaviruses, which include lymphocytic choriomeningitis virus (LCMV) (Stephensen *et al.*, 1991). A close relationship between the CH virus and LCMV is supported by the cross-reaction of several CH-specific sera with LCMV-Armstrong, which was implicated in several cases of asymptomatic LCMV infection in a research colony of rhesus monkeys (Armstrong and Lillie, 1934).

Prevention: Biosafety Level 2 practices, containment, equipment, and facilities are recommended for all activities using known or potentially infectious body fluids or tissues and animals known to be or potentially infected with LCMV; the same applies for the CH virus. LCMV may be present in blood, cerebrospinal fluid, urine, nasopharyngeal secretions, feces, and tissues of infected animals and people. Parenteral inoculation, inhalation, and contamination of mucous membranes or broken skin with infectious tissues or fluids from infected animals are common hazards, and aerosol transmission of LCMV is well documented. Laboratory-associated infections associated with LCMV-infected rodents are well documented, and naturally occurring infections occur in nonhuman primates (CDC/NIH, 1988). Two zoo veterinarians who cared for CH-infected callitrichids (at least one of whom was bitten by an infected animal) were found to be seropositive for CHV, but there is no history of clinical illness following exposure.

This disease is important not only because it is lethal to endangered species but also because a new type of epidemic primate hepatitis appeared without warning. Identification of the etiologic agent showed that the most probable source was mice (*Mus musculus*) which are common in zoos and are known to be hosts of LCMV. Finding seropositive, asymptomatic personnel with animal contact emphasizes the potential for transmission of viruses from primate to human animals.

D. Retroviral Diseases

The retroviruses are classified as type B, C, and D oncoviruses, foamy viruses, and lentiviruses. All are of significance in nonhuman primates, except the B type which is represented by the mouse mammary tumor virus (Desrosiers, 1988).

Type C oncoviruses include the murine, feline, and avian leukemia and sarcoma viruses. The human T cell leukemia viruses (HTLV-I and II), their simian counterpart (STLV-I), and the bovine leukemia virus form a distinct subgroup of type C oncoviruses. Type C oncovirus particles were demonstrated by electron microscopy in neoplastic tissues of a gibbon (*Hylobates lar*) with spontaneous lymphosarcoma and in naturally occurring fibrosarcoma in a woolly monkey (*Lagothrix* spp.) Experimental intradermal inoculation of this woolly monkey-derived simian sarcoma virus produced well-differentiated fibrosarcomas in marmosets (Rabin, 1978).

The prototype of type D oncoviruses is the Mason–Pfizer monkey virus, which was isolated from a rhesus monkey in 1969 (Chopra and Mason, 1969). This category includes the feline leukemia virus and macaque type D retroviruses which can produce a subclinical infection and slowly developing tumors, as well as immunosuppression. Simian retroviruses (SRV) have been associated with immunodeficiency and chronic wasting syndromes now called SAIDS, opportunistic infections, necrotizing gingivitis, and retroperitoneal fibromatosis (Daniel *et al.*, 1984; Desrosiers *et al.*, 1985; Marx *et al.*, 1984, 1985; Stromberg *et al.*, 1984).

Lentiviruses are not oncogenic and characteristically produce long-term, persistent infections which eventually lead to

chronic, debilitating diseases. This group includes HIV, which causes acquired immune deficiency syndrome (AIDS), and the simian immunodeficiency virus (SIV), as well as the classic ungulate lentiviruses (Maedi-Visna virus of sheep, caprine arthritis encephalitis virus, and equine infectious anemia virus) plus more recently isolated lentiviruses from cats (Pedersen *et al.*, 1987) and cattle (Gonda *et al.*, 1987). It should be noted that SAIDS can be caused by both type D retroviruses and lentiviruses (Benveniste *et al.*, 1986; Daniel *et al.*, 1987, 1988). These are distinctly different viruses so it is important to determine which virus is being described when reading the literature.

A number of SIV isolates have been obtained from several species of nonhuman primates at various primate facilities. Independent isolates of HIV and SIV vary from one another, to the extent that even independent isolates of HIV-1 from the same individual can vary (Hahn *et al.*, 1986). From the biosafety standpoint, an important feature of these viruses is their rate of mutation and the variability produced within a given isolate by *in vivo* transmission (Kestle *et al.*, 1988). A striking example of this phenomenon was the production of a new virulent strain of SIV_{SMM}, from a sooty mangabeys, by passage through a pig-tailed macaque. The parent virus, which readily establishes persistent infection in pig-tailed and rhesus macaques and in sooty mangabeys, causes progressive AIDS-like disease only in macaques. The new isolate ($SIV_{SMM\text{-}PBj14}$) infects all three species and causes acute disease characterized by bloody mucoid diarrhea and death within 12 weeks (Fultz *et al.*, 1989a). This demonstrates the potential for change in these agents that could adapt them for human infection.

The different infectivity, morbidity, and mortality rates *in vivo*, as well as different *in vitro* growth properties of SIV isolates, determine the relative value of each as models of HIV and optimal research use of each primate species. Macaques infected with SIV are good models for testing antiretroviral drugs because the effect on clinical illness can be evaluated. Macaques can also be infected with the HIV-2, which is antigenically similar to SIV, and these species are being used extensively for development of vaccines (Desrosiers *et al.*, 1989; Murphey-Corb *et al.*, 1989). Baboons (Anderson *et al.*, 1990) have also been used for testing the immunogenicity and safety of HIV subunit vaccines. Chimpanzees are the only primate animals that can be reproducibly be infected with both HIV-1 and HIV-2, although experimental infection of pig-tailed macaques (*M. nemestrina*) with HIV-1 has been reported (Agy *et al.*, 1992). These agents produce lasting infection in chimpanzees, but have not yet produced clinical disease in animals infected for 10 years. Chimpanzees have now been protected from HIV-1 infection for more than 1 year by vaccines (Girard *et al.*, 1991; Fultz *et al.*, 1992) and their primary use for human AIDS research is in vaccine development and safety testing, in experimental transfer of passive immunity, and in pharmocokinetic studies of antiviral drugs (Fultz *et al.*, 1989b).

The immediate cause of death in animals with an immune deficiency syndrome is usually the combination of opportunistic infections and bacterial sepsis associated with enterocolitis, diarrhea, and profound weight loss rather than the underlying retroviral infection. Personnel in contact with these animals are at risk from secondary agents (Gardner *et al.*, 1984) as well as the underlying virus.

Until 1992 there was no report of human illness or infection related to either SIV or SRV even though, retrospectively, it became known that these retroviruses have been responsible for spontaneously occurring disease in macaques at several primate centers for at least 20 years (Stowell *et al.*, 1971; Holmberg *et al.*, 1978; Lowenstein *et al.*, 1988). However, two suspected SIV human infections have been reported. One was a laboratory worker who suffered a needle stick injury while handling a SIV-infected monkey. This individual developed antibodies approximately 3 months after the injury, but the level of antibody declined over 2 years; no virus was ever isolated and PCR testing detected no viral sequences. These results indicate that the infection may have been cleared (Khabbaz *et al.*, 1992). The second was a laboratory worker who handled SIV-infected blood products without gloves while suffering from severe dermatitis on the hands and forearms. This individual developed antibodies to both SIV and HIV-2, which are very closely related, and levels continued to rise for 2 years, suggesting chronic infection. These cases emphasize the need for strict adherence to recommended guidelines for working with SIV because proper practices were not observed in each situation. In the dermatitis case, the individual was working with clinical specimens without gloves. Although the needle stick did penetrate a glove, the contaminated needle had been separated from the vacutainer holder before disposal (CDC, 1992b).

Prevention: Transmission is by parenteral inoculation, droplet exposure of mucus membranes, and contact exposure of broken skin; therefore, Biosafety Level 2 is recommended for handling monkeys with SIV infection. However, Biosafety Level 3 practices and equipment are recommended for work with purified virus and cultures of lymphocytes or tissues from animals infected with SIV and HIV-1 or HIV-2 (CDC, 1987c). Additional guidelines to minimize the potential risk of SIV transmission to laboratory workers and animal handlers have been formulated (Lairmore *et al.*, 1989).

E. Poxvirus Infections

Five nonhuman primate diseases caused by poxviruses also produce human disease. Monkeypox virus, which is related to smallpox virus, causes a generalized, sometimes febrile, illness characterized by proliferative vesicular skin lesions that become pustular and ulcerated. Many human cases of monkeypox infection have been reported including several that were fatal in African children (Breman *et al.*, 1980; Espana, 1971). Contact with monkeys is assumed to have been responsible for these naturally occurring human cases, and it has been speculated that monkeypox might be a reservoir for smallpox.

Benign epidermal monkeypox, also known as OrTeCa, is caused by a virus identical to the causative agent of tanapox in African children (Downie *et al.*, 1971) and is serologically related to the virus that causes Yaba-like disease (YLD) (Whitney, 1976). The disease caused by these two agents is characterized by multiple raised epidermal plaques, up to 1.5 cm in diameter. Although infections caused by these viruses will spread through colonies of susceptible monkeys, they are not life threatening to the animals. These infections are important, however, because they may produce disease in animal contact personnel. During one outbreak of YLD in a monkey colony, 11 handlers became infected. The monkeys developed no symptoms other than 2- to 4-cm, soft-centered, tumor-like dermal lesions. The disease ran its course in about 2 weeks. On the other hand, in the infected persons, all of which followed monkey-related trauma, the dermal lesions were accompanied by lymphadenopathy and high fever (Hull, 1969a).

Yaba poxvirus is an oncogenic virus first isolated from tumor tissue collected during an epizootic of self-limiting subcutaneous histiocytomas in a rhesus monkey colony at Yaba, Nigeria. The lesions of Yaba virus infection, experimentally produced in human volunteers by intradermal injection and accidentally in laboratory workers by needle stick, were similar to those seen in monkeys. Nodular lesions appear 5–7 days after intradermal injection, attain a maximum size of 2–5 cm in 3 weeks, and regress spontaneously by 6–8 weeks postinoculation. However, it is not uncommon for new tumors to form while others are in various stages of progression, so it may take several months for all tumors to regress completely (Griesemer and Manning, 1973).

Molluscum contagiosum, a human poxvirus that produces papular to papillomatous skin lesions, has been reported in chimpanzees (Whitney, 1976).

Smallpox has been eradicated as a worldwide public health problem, but recent use of vaccinia virus as a vector for genetically engineered vaccines against other viruses such as HBV and HIV has brought the potential for poxvirus exposure into many primate research facilities (Van Eendenburg *et al.*, 1989).

Prevention: Biosafety Level 2 precautions are recommended. All transmission of simian poxvirus diseases from nonhuman primates to their handlers, in the laboratory setting, has been by accidental needle stick; experimental intradermal injection; and bites, scratches, or other trauma. However, experimental aerosol transmission of Yaba virus has been reported (Griesemer and Manning, 1973).

Poxviruses may be present in lesion fluids or crusts, respiratory secretions, or tissues of infected hosts. Ingestion, parenteral inoculation, and droplet or aerosol exposure of mucous membranes or broken skin with infectious fluids or tissues are the primary hazards to laboratory and animal care personnel. Some poxviruses are stable at ambient temperature when dried and may be transmitted by fomites (CDC/NIH, 1988). Therefore, in addition to strict adherence to universal precautions, special care to disinfect work surfaces, cages, and the like should be exercised.

All people entering laboratory or animal care areas where activities with vaccinia, monkeypox, or other orthopox viruses are conducted should have documented evidence of satisfactory vaccination within the preceding 3 years (CDC, 1991c). Vaccine for administration to laboratory personnel at risk may be obtained by special request from the Drug Service, Scientific Resources Program, National Center for Infectious Diseases, Center for Disease Control, Atlanta, Georgia.

F. Other Viral Infections

Since it appears that all known human viruses can infect one or more nonhuman primate species, it would be impossible to list all that have been found naturally occurring or given experimentally to primate animals. However, representative members of the major virus groups that have not been previously discussed should be mentioned.

1. Measles and Influenza

The measles virus is a member of the human myxoparamyxovirus group, which also includes mumps and influenza viruses (Muchmore and Swack, 1978; O'Brien and Tauraso, 1973). Simian virus 5 (SV5), one of the most common contaminants of both African and Asian monkey cell cultures, and SV41 are simian viruses of this group (Hull, 1969a).

Measles infection in monkeys, as demonstrated by experimental infection, presents various clinical syndromes, ranging from asymptomatic development of a positive serologic titer to the entire clinical syndrome of fever, leukopenia, conjunctivitis, Koplik's spots on labial and buccal mucosa, and, finally, a red maculopapular rash that covers most of the anterior body surface. Studies have shown that rhesus monkeys from India are free of measles in their native habitat, but often all members of a newly imported group will show serologic or clinical evidence of infection within a few weeks after importation (Hall *et al.*, 1971). It is hypothesized that this highly contagious disease, transmitted by aerosols, is spread throughout a colony of newly captured animals from one or more animals infected by human exposure. Measles has been such an important cause of morbidity and occasional mortality in newly imported monkeys (Whitney, 1976) that some importers have given the monkeys prophylactic injections of human ISG upon arrival in this country. Since sporadic outbreaks of measles continue to occur in the United States, despite mandatory vaccination for preschool children, the potential for infection of animals by newly infected people, or vice versa, remains a threat.

Influenza, experimental and naturally occurring, has been reported in various species of nonhuman primates, as has horizontal transmission in baboons following experimental infection (Kalter and Heberling, 1973). Data obtained from a 10-month study of influenza infection in baboons, chimpanzees, cynomolgus monkeys, and a group of human volunteers engaged in working with these animals suggested that the nonhuman pri-

mates were not a potential reservoir, but that they reacted to the virus present in the area as do humans (Kalter and Heberling, 1973).

Prevention: There are no reported cases of transmission of measles or influenza viruses to people from nonhuman primates, but there is evidence that the virus passes from humans to animals. Effective vaccines are available for measles, mumps, and influenza, all of which can be transmitted from human to nonhuman primates, and vice versa; therefore, all persons who have contact with primates should be immunized against these agents so that they do not infect susceptible research animals.

2. Picornavirus Infections

Viruses belonging to the family Picornaviridae include polio viruses, coxsackieviruses, other enteroviruses, and rhinoviruses. The susceptibility of nonhuman primates to poliovirus was reported in 1909, and the use of monkeys and their tissues for development and production of polio vaccines was responsible for the importation of many nonhuman primates before domestic breeding supplies became available. Naturally occurring poliomyelitis has been seen in both wild and captive groups of chimpanzees (Douglas *et al.*, 1970; Guilloud *et al.*, 1969), and coxsackievirus infection of a laboratory-born infant chimpanzee has been reported (Kelly *et al.*, 1978).

Human rhinoviruses have been found naturally occurring in chimpanzees and have been studied in this species and in gibbons (Dick and Dick, 1968; Pinto *et al.*, 1972).

Prevention: Precautionary measures, over and above Biosafety Level 2 procedures, are dependent on the mode of transmission of each agent, with universal precautions for bloodborne organisms, enteric precautions for those with fecal–oral spread, and respiratory precautions for those with aerosol potential.

3. Arbovirus Infections

Arboviruses, or arthropod-borne viruses, include yellow fever and dengue, which are discussed with their biohazard potential as hemorrhagic fever viruses. Many others have been found and studied in nonhuman primates, including Chikungunya, which is maintained in vervet monkeys and baboons and is transmitted by biting flies and mosquitoes (DeMoor and Steffens, 1970; McIntosh *et al.*, 1978); Kyasanur Forest disease spread by ticks in India (Rajagopalan and Anderson, 1971); and West Nile and Rift Valley fevers (Pogodina *et al.*, 1983; Davies and Onyango, 1978).

Prevention: Biosafety Level 3 or 4 is recommended for research activities with these arboviruses because of the demonstrated high potential for laboratory infections and severe consequences should infection occur (CDC/NIH, 1988). What must be remembered is that agents of this class, like the Ebola virus, could be brought in with any shipment of animals from the wild. Therefore, quarantine procedures should provide for thorough evaluation of the cause of death or illness of all newly imported animals. Even though these viruses are spread by arthropods in the wild, it is essential to keep in mind that even arboviruses can be transmitted in the laboratory, not only by accidents such as needle sticks, but also by the natural route in areas where the vectors are found, if animals are housed outdoors or in inadequately screened facilities.

4. Rubella

Rubella is a togavirus that is transmitted from mother to fetus and causes abortion or fetal damage in nonhuman primates, just as it does in people; it can also be fatal in young animals. Therefore, the concern in primate facilities is not the zoonotic potential of rubella, but the potential for handlers to bring infection to the animals.

Prevention: Optimally, all people should be immunized against the rubella virus for overall public health and, minimally, all health-care workers, including those involved with research using nonhuman primates, should have documented proof of adequate immunization by serologic testing or vaccination. Clinical diagnosis of rubella is unreliable and should not be considered in assessing immune status. People who do not have a protective titer should be immunized. The only contraindications for immunization are pregnancy or recent (within 3 months) ISG injection (CDC, 1991a).

5. Rhabdovirus Infections

Rabies virus and vesicular stomatitis virus are rhabdoviruses, both of which have been found naturally occurring in nonhuman primates. There were 10 documented cases of simian rabies reported in the United States from 1929 to 1970, but rabies may be a more frequent disease of nonhuman primates than commonly thought (Richardson and Humphrey, 1971). Rabies remains endemic in all countries from which nonhuman primates are imported, and ample opportunity exists for exposure of both free-ranging and recently trapped animals prior to export.

The possibility of a long incubation period for rabies infection must be kept in mind. A rhesus monkey experimentally inoculated with a virulent strain of rabies at CDC did not develop the furious form usually seen 15–35 days postinoculation, but appeared normal in all respects until the 105th day, when it was found comatose. This means that an animal infected with rabies could pass the traditional 90-day quarantine with no evidence of disease and that animals dying of inapparent causes during (or even shortly after) quarantine might have rabies. The possibility of rabies should be considered at necropsy of such animals because it will not be detected unless brain tissue is specifically subjected to fluorescent antibody or other definitive examination (Richardson and Humphrey, 1971).

Prevention: The tissues and excretions of naturally or experimentally infected animals are potential sources of human exposure; the highest titers are in the central nervous system, salivary glands, and saliva. Preexposure vaccination should be offered to individuals working with newly reported primates in

quarantine and is recommended for those working with rabies virus or known infected animals. Accidental parenteral inoculation, cuts or sticks with contaminated laboratory equipment, bites from infected animals, and exposure of mucous membranes or broken skin to infectious droplets of tissue or fluids are the most likely means of exposure. Wounds should be thoroughly washed with soapy water and deep puncture wounds flushed via catheter; cautery and suturing are not recommended. Both human diploid cell vaccine and rabies immune globulin (RIG) should be given immediately. If RIG is not available, antirabies serum (equine origin) is recommended (Berkow and Fletcher, 1987).

Although it may not be feasible to open the skull or remove the brain within a biological safety cabinet, it is pertinent to wear heavy protective gloves, to minimize the chance of injury from instruments or bone fragments, and to wear a face shield to protect the mucous membranes of the eyes, nose, and mouth from exposure to droplets or tissue fragments. If one is using a Stryker saw to open the skull, care should be taken to avoid striking the brain with the saw blade (CDC/NIH, 1988). These recommendations apply to all nonhuman primate necropsies.

6. Adenovirus Infections

Simian adenoviruses have been associated with infections of the respiratory tract (Boyce *et al.*, 1978), eye, pancreas (Chandler *et al.*, 1974), and urinary tract (Asher *et al.*, 1978).

These viruses have not been of major zoonotic importance, but there is one report of human infection with a simian adenovirus that illustrates a potential source of accidental laboratory infection. A technician, attempting to remove an obstruction that developed in the needle on a syringe filled with SV23 during a series of animal inoculations, sprayed a drop of virus into the eye of one of the operators. Severe conjunctivitis developed, spread to the other eye, and persisted for 5 weeks, but recovery was complete without sequelae (Hull, 1969a).

Prevention: The accident just described reinforces the recommendation for wearing goggles, glasses, or a face shield when handling infectious agents. It also emphasizes the importance of always having an alcohol sponge, sterile cotton ball, or gauze around the tip of the needle on a syringe of infectious material, even for tapping out the bubbles, while exercising extreme caution to prevent needle stick, the most common laboratory accident.

7. Papovavirus Infection

Papovavirus-like particles were found in tissue taken from an oral mucosal lesion of focal epithelial hyperplasia in a chimpanzee. These striking nodular elevations were found primarily on the mucosa of the lips, but were also present on the buccal mucosa and tongue. In the outbreak described, lesions were found in 16 of 45 chimpanzees examined. At the same time, several animal caretakers reported warts (verucca vulgaris) on the knuckles of fingers that were sometimes bitten by young chimpanzees. Since the papillomavirus that causes human warts is a papillomavirus from the family Papovaviridae, it was speculated that the two were related, but this was not confirmed (Hollander and van Noord, 1972).

Prevention: If, indeed, the human warts were related to the chimpanzee oral lesions, wearing gloves should have prevented spread of the virus. If caretakers accustomed to having chimpanzees bite their fingers suffered no more serious consequence than warts, they were fortunate because even small nonhuman primates such as squirrel monkeys and marmosets can inflict serious bite wounds.

8. Spongiform Encephalopathy Viruses or Prions

The causative agents of the spongiform encephalopathies known as Creutzfeldt-Jakob disease and Kuru in people, scrapie in sheep, and others are in a class by themselves (Brown *et al.*, 1984). They have an extraordinarily long incubation period that ranges from months to decades so they have been known as "slow viruses" or "unconventional viruses" and, most recently, as "prions." This name was derived from the "protein-like infectious particles" found in the brain tissue infected with scrapie (Prusiner, 1982). These agents are extremely resistant to all standard means of sterilization: acetone, alcohol, hypochlorite, iodine, peroxide, permanganate, formaldehyde, glutaraldehyde, ethylene oxide, β-propiolactone, nucleases, proteases, detergents, ultraviolet radiation, ultrasonic energy, or even steam autoclaving for less than an hour (Brown *et al.*, 1982; Chatigny, 1989; Chatigny and Prusiner, 1980).

The human agents have been transmitted to several species of nonhuman primates by intramuscular injection (Gibbs and Gadjusek, 1976), but there are no reported cases of human infection from work with them.

Prevention: Biosafety Level 2 conditions and universal precautions are recommended for working with prion-infected nonhuman primates.

III. BACTERIAL DISEASES

A. Systemic Infections

1. Tuberculosis

Tuberculosis is the most important bacterial disease of nonhuman primates because of its ubiquitous and insidious nature and its ability to spread rapidly. Although it is usually thought of as a respiratory disease, tuberculosis may be found in any organ, so it should be regarded as a systemic infection.

Most of the tuberculosis seen in nonhuman primates is caused by the acid-fast bacillus *M. tuberculosis*, but many different strains have been recovered, including *M. bovis* (Renner and Bartholomew, 1974), *M. avium* (Smith *et al.*, 1973), *M. kansasii*

(Valerio *et al.*, 1978), *M. scrofulaceum* (Renquist and Potkay, 1979), and *M. intracellulare* (CDC, 1973).

Tuberculosis occurs in all primate species, but susceptibility varies. Young macaques are the most susceptible and New World monkeys the least, but there have been several outbreaks even in the latter. The human infectious dose (ID_{50}) of *M. tuberculosis* is <10 bacilli, and it is has been postulated that only one bacillus will infect a rhesus monkey. In young macaques, tuberculosis spreads rapidly throughout a closed colony until all become infected (Keeling *et al.*, 1969). Older rhesus monkeys, baboons, and apes usually have a slower form of the disease, similar to that seen in people (Whitney, 1976).

Although documented cases are hard to substantiate, naturally or experimentally infected nonhuman primates have been suspected to be the source of some human infections. The CDC estimated the annual rate of tuberculin conversion for individuals having direct contact with nonhuman primates to be 60 to 100 times that of the general population in 1970 and 1971. During the 1970s the incidence of tuberculosis in the United States dropped and routine tuberculin testing and eradication of infected primate animals became common practice in research facilities. However, in 1978 the tuberculin conversion rate among personnel working with infected animals was still reported to be more than 20 times greater than that of the general population (Kaufmann and Anderson, 1978). Tuberculosis in the United States began to increase again in the 1980s, and multidrug-resistant strains appeared in the early 1990s (CDC, 1992a). Transmission from man to monkey has been reported (Cappucci *et al.*, 1972), so the need for conscientious monitoring of people working with primate animals remains essential to keep the spread from getting into animal colonies.

Tuberculosis infections are acquired primarily from aerosols via the respiratory route or the gastrointestinal route. In addition to the potential for exposure through these common routes, laboratory workers have potential for contact with the blood, sputum, excreta, cerebrospinal fluid, exudates from lesions, and tissues of infected animals. Since tuberculosis can be transmitted by blood, careless injection procedures or failure to sterilize items such as tattoo needles and thermometers between animals could result in the spread of disease in a colony (Whitney, 1976). Tubercle bacilli may even survive in heat-fixed smears (Allen, 1981) and in inadequately formalin-fixed tissues.

Prevention: Prevention of exposure is of paramount importance in controlling tuberculosis in primate facilities. The quarantine of animals coming into a facility must be long enough to detect any recently acquired infections that have not yet produced a delayed hypersensitivity response in the host. Even animals that are transported from one stable colony to another could be exposed en route and should be quarantined with tuberculin testing every 2 weeks for 90 days before being incorporated into the new stable colony. Stable colony animals should be tuberculin tested quarterly (Henrickson, 1984).

Tuberculin-negative animal contact personnel should be Mantoux tuberculin skin tested routinely every 6 months with 5 tuberculin units of purified protein derivative (CDC, 1990b). Tuberculin-positive people should be monitored medically whenever they have respiratory symptoms that persist longer than those usually seen with transient viral upper respiratory infections.

Human tuberculin converters should be referred to a physician for follow-up with radiographs, cultures, or other diagnostic procedures and treatment (Muchmore, 1976). People whose jobs require animal contact should be assigned other duties until all diagnostic tests are completed and they are considered free of infection that could shed tubercle bacilli. If no evidence of infection is found, a 1-year course of prophylactic therapy with isoniazid is recommended. However, many people cannot tolerate this drug, and the liver enzymes, aspartate and alanine aminotransferase and γ-glutamyltransferase, must be monitored at least once a month for evidence of drug-induced hepatitis. Since isoniazid eliminates circulating bacilli, it is thought that recent converters, with no visible focus of infection, can be permitted to return to animal contact work after a minimum of 2 weeks on isoniazid, but they should be encouraged to complete the full course for prophylaxis unless side effects necessitate stopping treatment.

Animals with overwhelming infection may be anergic and give a negative tuberculin skin test (Corcoran and Jaax, 1991), so necropsy of all animals that die during quarantine should be performed with tuberculosis in mind and with not less than Biosafety Level 2 precautions and containment equipment.

Biosafety Level 3 practices and facilities are recommended for animal studies using nonhuman primates that are naturally or experimentally infected with *M. tuberculosis* and *M. bovis* (CDC/NIH, 1988). Therefore, animals that are tuberculin positive should be killed if this level of containment is not available. Multidrug treatment may be considered but only if appropriate isolation and containment facilities can be provided and accurate detection of infection is possible (Ward *et al.*, 1985; Wolf *et al.*, 1988). All animals that have been housed in the same air supply areas as infected animals should begin a new 90-day quarantine period with biweekly tuberculin testing every time a new reactor is identified. All previously negative contact personnel should be tuberculin tested again and access personnel kept to a minimum.

2. Leprosy

Spontaneously occurring infection with *Mycobacterium leprae*, which causes the primarily human disease leprosy (Hansen's disease), has been seen in chimpanzees (Meyers *et al.*, 1984; Gormus *et al.*, 1991), armadillos (*Dasypus* spp.) (Walsh *et al.*, 1975), and a mangabey (*Cercocebus*) (Meyers *et al.*, 1980). This agent cannot be grown satisfactorily in artificial media and attempts to develop an animal model have shown that armadillos are overwhelmed by the organism so they are not suitable for efficient investigation of epidemiology, immunology, and therapy. However, mangabeys, rhesus monkeys,

and African green monkeys (*C. aethiops*) now appear to be acceptable animal models for leprosy.

Human-to-human transmission of leprosy has been reported following an accidental needle stick in a surgeon (Marchoux, 1934) and the use of a presumably contaminated tattoo needle (Parritt and Olsen, 1947). The infectious organisms can be found in tissues and exudates from lesions of infected individuals. Therefore, infected animals can pose a risk to their human handlers and failure to sterilize instruments between animals could spread infection among them.

Prevention: Biosafety Level 2 practices, containment, and facilities are recommended. Special care should be taken to avoid accidental transmission by the exposure of skin and mucous membranes to infectious materials, and extreme caution should be employed when using sharp instruments, especially needles, when working with infected animals.

3. Salmonellosis

Salmonellosis is a systemic infection, and infectious gram-negative rods can be found not only in feces but also in blood and urine. The asymptomatic carrier state exists in nonhuman as well as human primates, so apparently healthy animals can be shedding the bacteria and stress may precipitate the recurrence of active disease (Whitney, 1976). It is important to remember that animals with *Salmonella*-associated diarrhea, with or without fever, may have infectious organisms present in other body fluids. In addition, the ability of most *Salmonella* species not only to survive but also to multiply for considerable periods in moist organic material is an important epidemiologic feature. Where animals are held in outdoor corrals or bedding is used, this potential for contamination of soil and vegetation should be an important consideration.

Although salmonellosis is known to be a laboratory hazard (Pike, 1976), studies have shown that there is little risk of infection to laboratory personnel and animal handlers exposed to nonhuman primates (Gerone, 1983).

Prevention: Biosafety Level 2 practices, containment, and facilities with enteric precautions are recommended. The licensed typhoid vaccine provides only partial protection, but may be useful for people who regularly work with cultures or materials known to contain *S. typhi* (Blaser *et al.*, 1980a, b); however, it is not currently recommended for individuals working with nonhuman primates.

4. Tetanus

Clostridium tetani is an anaerobic, gram-positive, spore-forming bacillus that causes tetanus by producing a potent exotoxin that is intensely toxic for humans and animals when it is formed in tissues or when injected. The organism is found in soil and in the intestinal tracts of humans and other animals. Tetanus was a major cause of death in the free-ranging rhesus monkey colony on Cayo Santiago in Puerto Rico, before an immunization program was instituted.

Prevention: Tetanus organisms are ubiquitous, so it is recommended that all persons should be vaccinated. Since the potential for receiving contaminated puncture wounds from bites and scratches is greater when working with primate animals than in many other occupations, personnel records should show that all employees have received a primary tetanus immunization course of three doses followed by a booster every 10 years. Up to date records should be available and provided to health-care professionals treating work-related wounds so that appropriate tetanus coverage can be given.

5. Other Generalized Bacterial Infections

Neisseria gonorrheae and *N. meningitidis* are human pathogens that have known biohazard potentials for laboratory workers (CDC/NIH, 1988). Both have been found naturally occurring (Brown *et al.*, 1973) and experimentally induced in chimpanzees (Kuhn, 1971).

Prevention: These agents may be present in conjunctival, urethral, and cervical exudates; synovial fluid; urine; feces; and cerebrospinal fluid. Accidental parenteral inoculation and direct or indirect contact of mucous membranes with infectious clinical materials are the primary laboratory hazards, so universal precautions under Biosafety Level 2 conditions should be observed.

Pseudomonas pseudomallei is endemic to southeast Asia and may be found elsewhere as a normal inhabitant of soil and water. Many natives of this area have subclinical infections. However, it can produce a severe and often fatal disease characterized by disseminated or localized abscesses in humans and animals. Melioidosis has been reported in several macaque species (*M. mulatta, M. arctoides, M. nemiestrina*), in an orangutan in Australia, and in one chimpanzee (Kaufmann *et al.*, 1970). A striking feature of melioidosis is that it may not become clinically apparent for months or years after an individual leaves the disease-endemic area. One group of infected monkeys had been in the United States for 6 months to 3 years before the disease became clinically apparent. The source of infection in the 4-year-old chimpanzee was not determined. However, it was hypothesized that the organism was transmitted by animal caretakers who also worked with infected macaques in the same colony (Butler *et al.*, 1971).

Prevention: *P. pseudomallei* may be present in sputum, blood, wound exudates, and various tissues, depending on the site of localization of infection. Gloves should be worn when handling and during necropsy of infected animals and when direct skin contact with infectious materials is likely (CDC/NIH, 1988).

B. Gastrointestinal Bacterial Infections

Diarrhea is the most common cause of morbidity and mortality in nonhuman primates, and the etiologic agents isolated represent all of the pathogenic human enteric bacteria.

1. Shigellosis

Infection with the gram-negative, nonspore-forming bacilli of *Shigella* spp. is extremely common among captive nonhuman primates. The most frequently found species is *S. flexneri*, but *S. sonnei, S. boydii*, and *S. dysenteriae* have all been isolated (T-W-Fiennes, 1967). Shigellosis is the most frequently identified cause of diarrhea in these animals (Irving, 1974) and is a significant zoonotic disease (Mulder, 1971; Pike, 1976).

Acute shigellosis in nonhuman primates is often fulminant and fatal. It is characterized by diarrhea with mucus and blood, prostration, edema of the face and neck, emaciation, and, frequently, prolapse of the rectum. Animals that recover from an episode often remain asymptomatic carriers and, although they may have negative fecal cultures, they may also have recurrent acute episodes in times of stress (Whitney, 1976).

The disease in people, like that in nonhuman primate, varies from the completely healthy asymptomatic carrier state to a severe bacillary dysentery syndrome with bloody mucoid diarrhea, abdominal cramping, tenesmus, anorexia, and weight loss. The disease is usually more severe in children than in adults and may be fatal.

Shigella organisms, which may be present in the feces and rarely in the blood of infected individuals, are spread primarily by the fecal–oral route and also by parenteral inoculation. The infectious dose is very small: the ID_{25}–ID_{50} of *S. flexneri* for humans is reported to be approximately 200 organisms (Wedum *et al.*, 1972). Very minimal contact between infected animals and humans has been incriminated in fatal disease (T-W-Fiennes, 1967), and asymptomatic animals that are shedding shigellae have been a particular threat to pet store proprietors just as they are to laboratory workers and laboratory animals (Fox, 1975).

Prevention: Animal Biosafety Level 2 facilities and practices are recommended for activities with experimentally or naturally infected animals. Access of personnel to areas where there are animals with shigellosis should be minimal, and those who work with infected animals should especially observe enteric precautions with good personal hygiene and thorough hand washing. Strict quarantine of newly imported nonhuman primates is essential. Animals with clinical disease should be further isolated for treatment and must have repeated negative stool cultures before being returned to the colony. It has also been recommended that all newly arrived nonhuman primates should be screened for *Shigella* infection so that asymptomatic carriers can also be treated to prevent spread in the colony.

2. Campylobacteriosis

Campylobacteriosis is a disease with moderate to severe enterocolitis caused by *Campylobacter* spp. which are small gram-negative, nonspore-forming, slender, spirally curved, rod-shaped bacteria. The most significant member of this genus is *C. jejuni*, a motile form with a single polar flagellum (Snibert, 1974) that was formerly classified as a *Vibrio* and is now known as *Helicobacter*.

In healthy human adults, the disease is usually a mild, self-limiting enteritis characterized by fever, malaise, dizziness, myalgia, abdominal pain, and watery, bile-stained, malodorous diarrhea (Butzler and Skirrow, 1979), which is usually attributed to food poisoning or the "flu." However, campylobacteriosis is an important zoonosis because it can cause severe and even fatal disease in young children (Coffin *et al.*, 1982) and immunodeficient adults, and because of the increasing prevalence of infection. Transmission is usually by the oral route and viable *Campylobacter* organisms are found in laboratory animals, including nonhuman primates (Renquist, 1987), companion animals (Blaser *et al.*, 1978, 1982), poultry (Deming *et al.*, 1987), and milk (Vogt *et al.*, 1984); they can survive even in 6° C stream water (Terzieva and McPeters, 1991). Human *C. jejuni* infection has been linked to pet animals and transmission from laboratory primates has been reported but not thoroughly documented. However, the zoonotic potential of these organisms in a research laboratory setting has been conclusively demonstrated by serotyping and restriction endonuclease DNA analysis of the strains found in a laboratory animal technician with enteritis and animals he was attending (Fox *et al.*, 1989).

Prevention: Biosafety Level 2 practices and facilities with enteric precautions are recommended for housing and working with animals naturally or experimentally infected with organisms transmitted by the fecal–oral route (CDC/NIH, 1988).

3. Other Zoonotic Enteric Bacteria

Yersinia enterocolitica causes yersiniosis, a common human intestinal disease marked by diarrhea, enteritis, pseudoappendicitis, ileitis, erythema nodosum, and sometimes septicemia (Skavlen *et al.*, 1985) or acute arthritis. *Y. pseudotuberculosis* causes diarrhea and can produce mesenteric lymphadenitis. These gram-negative, unencapsulated ovoid- to rod-shaped organisms are hard to isolate on routine rectal culture and may be overlooked, but they are found in the intestine and lymph nodes of both sick and healthy primates, including humans. Spontaneous disease related to both *Yersinia* spp. has been reported in groups of several nonhuman primate species (Rosenberg *et al.*, 1980; Bronson *et al.*, 1972; Buhles *et al.*, 1981; Poelma *et al.*, 1977).

Prevention: Biosafety Level 2 practices and facilities with enteric precautions are recommended for housing and working with animals naturally or experimentally infected with organisms that are spread via the fecal–oral route (CDC/NIH, 1988). It is noteworthy that a study to determine the potential of cockroaches (*Periplaneta americana*) for carrying enteropathogenic organisms showed that cockroaches trapped in the primate housing area of a major zoo did, indeed, contain at least one strain of *E. coli, Streptococcus fecalis*, and *Paracolon* spp. identical to those isolated at the same time from nonhuman primates housed there (Greenberg and Sanati, 1970). This reinforces the recommendation for keeping animal housing areas free of vermin.

C. Respiratory Bacterial Infections

Respiratory disease is second to gastrointestinal infection as the most common cause of morbidity and mortality in nonhuman primates. Among the bacteria commonly found causing pneumonia or upper respiratory tract infections in nonhuman primates are *Streptococcus pneumoniae, Bordetella bronchiseptica* (Graves, 1968), *Pasteurella multocida, Staphylococcus aureus, Klebsiella pneumoniae* (T-W-Fiennes, 1967; Kageruka *et al.*, 1971), and *Hemophilus influenzae*, with *E. coli*, streptococci, and *Staphylococcus aureus* as secondary invaders (Good and May, 1971; Henrickson, 1984). *Pseudomonas, Proteus*, and *Corynebacteria* infections have also been reported, so it is evident that nonhuman and human primates share the same pathogenic respiratory tract flora.

Prevention: Surgical-type masks, which should be routinely worn by personnel entering or working in rooms with nonhuman primates, may serve as physical barriers and minimize aerosol droplet spread between personnel and animals (Dineen, 1971). However, when working with animals known to have respiratory infection, it is preferable to wear HEPA-type face masks. A facility ventilation system that provides nonrecirculating air at 10 or more changes per hour may also be of value in reducing the spread of respiratory diseases within individual rooms as well as throughout the facility.

IV. SPIROCHETAL DISEASES

Spirochetes of the genus *Treponema* are the causative agents of syphilis (*T. pallidum*), yaws (*T. pertenue*), and pinta (*T. carateum*). Syphilis has a characteristic primary lesion (chancre) at the site of infection, followed by a secondary granulomatous skin eruption; tertiary disease may develop many years later in the central nervous system or any organ (Elsas *et al.*, 1968). Yaws is a tropical disease characterized by a primary cutaneous lesion, followed by a granulomatous skin eruption, and sometimes by late destructive lesions of the skin and bones. Pinta is a dermatotropic disease of people found primarily in South and Central America, with immunologic similarities to syphilis, but lacking its generalized and serious consequences (Kuhn *et al.*, 1968).

Testing of many of the species of nonhuman primates from various geographic areas showed that chimpanzees and many species of African monkeys were positive for both screening and confirmatory tests for treponemal infection. Almost all of the positive animals were baboons, patas monkeys, and chimpanzees, all of which are of African origin. One study reported that 85% of monkeys from a yaws-endemic area in Africa had reactive fluorescent treponemal antibody absorption (FTA-ABS) tests; 15% of 250 normal chimpanzees had reactive FTA-ABS tests in another study. A *Treponema* strain with morphologic and immunologic characteristics identical to those of *T. pallidum* and *T. pertenue* was derived from popliteal lymph nodes of a baboon from Guinea (Fribourg-Blanc and Mollaret, 1969). The epidemiologic data support the concept that treponematosis is enzootic among nonhuman primates (Felsenfeld and Wolf, 1971; Levine *et al.*, 1970) and there are chimpanzees, imported from the wild before 1975 and not experimentally exposed to Treponema, that were *Treponema pallidum* immobilization (TPI) test positive after years in captivity.

Borrelia recurrentis, the causative agent of louse-borne relapsing fever, has been experimentally transmitted to *C. aethiops* monkeys by intraperitoneal inoculation of blood from human patients. These animals developed the same relapsing febrile disease and histopathologic changes found in human patients (Judge *et al.*, 1974).

Prevention: Treponemes may be present in materials collected from primary and secondary cutaneous lesions and in blood. Accidental parenteral inoculation and contact of mucous membranes or broken skin with infectious clinical materials are the primary hazards to laboratory personnel. Biosafety Level 2 conditions and universal precautions are recommended for work with animals infected by these organisms (CDC/NIH, 1988).

People are susceptible to infection with most members of the genus *Leptospira* that are found in many research animal species, including dogs, rodents, and nonhuman primates. Occurrence of severe leptospirosis in a breeding colony of squirrel monkeys has been reported (Perolat *et al.*, 1992). Leptospirosis, like syphilis, is a great imitator and may produce a variety of clinical symptoms. Therefore, cases may escape detection if serologic tests for leptospirosis are not included in the battery of tests done for "fevers of unknown origin" (Lennette, 1973). *Leptospira* organisms may be present in urine, blood, and tissues of infected animals and humans. Ingestion, accidental parenteral inoculation, and direct and indirect contact of skin or mucous membranes, especially with urine, are the primary laboratory hazards (CDC/NIH, 1988). The prevalence of *Leptospira* organisms in the urine of mice is sufficient reason for keeping animal feed rooms vermin free.

V. MYCOPLASMAL DISEASES

The universal distribution of mycoplasmas in animals and people is well documented (Lennette, 1973). Seventy strains of *Mycoplasma* from oropharyngeal and urogenital swabs and tissues have been reported from chimpanzees and African monkeys (Kalter, 1972; Barile, 1973). *Mycoplasma pneumoniae*, a major human pathogen, has been reported as naturally occurring and has been studied in rhesus monkeys (Friedlander *et al.*, 1976).

Mycoplasmas are frequent contaminants of cell cultures, so there is controversy over recent reports that mycoplasmas may potentiate the cytopathic effect of the HIV (Lo *et al.*, 1991).

Ureaplasma urealyticum (formerly known as T-mycoplasma), a mycoplasma that infects the genital tract, has been associated with spontaneous abortion and infertility in women and urethritis in men. Simian species reported to harbor ureaplasmas include talapoins, patas monkeys, macaques, squirrel monkeys, marmosets, and chimpanzees (Swenson and O'Leary, 1977).

Prevention: The mycoplasmas included in this discussion have not been reported as laboratory hazards; however, the fact that both human and nonhuman primates can be infected means that they should be treated as potential biohazards and that Biosafety Level 2 conditions should apply.

VI. RICKETTSIAL DISEASES

Rickettsial organisms are a group of pleomorphic, rod-shaped, gram-negative bacteria that are parasitic in lice, fleas, ticks, and mites. They are transmitted to mammals by the bite of their arthropod hosts and cause typhus, tsutsugamushi, rickettsial pox, Rocky Mountain spotted fever, Q fever, and other diseases.

Newly captured cynomolgus monkeys (*M. fascicularis*) have serologic evidence of naturally acquired scrub typhus or tsutsugamushi, which is caused by *Rickettsia tsutsugamushi* (Kitaoka, 1972). Experimental infection of cynomolgus and silvered leaf monkeys (*Presbytis cristatus*) has shown that they are good models for studying this disease (MacMillan and Rice, 1985).

Coxiella burnetii, which causes Q fever in man, is the type species of this rickettsial genus. Antibodies to *C. burnetii* have been found in baboons and other nonhuman primates, so it is evident that these animals become infected with Q fever and, like almost all other wild animals, could be carriers (T-W-Fiennes, 1967).

Prevention: Rickettsiae are present not only in their arthropod vectors, but also in the blood, urine, feces, milk, and tissues of infected animal or human hosts. Parenteral inoculation and exposure to infectious aerosols and droplets are the most likely sources of infection to laboratory and animal care personnel. Tissues infected with Q fever may contain up to 10^9 organisms per gram, and the human inhalation ID_{25}–ID_{50} is 10 organisms. Nevertheless, Biosafety Level 2 is recommended for laboratory procedures other than those involving culture of the organisms, which should be done under Biosafety Level 3 (CDC/NIH, 1988).

VII. CHLAMYDIAL DISEASES

Chlamydiae are small, gram-positive, coccoid bacteria that resemble rickettsiae and were formerly classified as the psittacosis–lymphogranuloma–trachoma group of that order. A naturally occurring *Chlamydia* infection has been reported in *M. fascicularis* from the Philippines. Gross necropsy findings included pleuropneumonia and ulceration of the tongue and lips, and the diagnosis was based on histologic and serologic examinations. Epithelial cells from a tongue ulcer contained numerous reticulate bodies, elementary bodies, and cytoplasmic includion bodies, observed by light microscopy. Chlamydia-like particles were seen in the lung (Morita *et al.*, 1971). A number of experimental infection studies have been performed in macaques and baboons (DiGiacomo *et al.*, 1975).

Prevention: *C. psittaci* is found in tissues, feces, nasal secretions, blood, sputum, and tissues of infected individuals; *C. trachomatis* is found in genital, bubo, and conjunctival fluids of infected animals. Infections with psittacosis lymphogranuloma venereum and trachoma were, historically, the fifth most commonly reported laboratory-associated bacterial infection. This was primarily due to the high incidence of psittacosis from handling infected birds before 1955, which emphasizes the contagiousness of these organisms.

Universal precautions with Biosafety Level 2 practices are recommended for all activities except those with high potential for droplet or aerosol production involving production quantities or concentration of infectious materials, for which Biosafety Level 3 precautions are advised (CDC/NIH, 1988).

VIII. MYCOTIC DISEASES

Fungal diseases are not common in nonhuman primates, but representatives of all of the major human mycotic pathogens have been found in one or more species and are transmissible to humans.

Among the fungi that cause systemic infections are *Aspergillus* spp., *Nocardia caviae, Nocardia asteroides, Histoplasma capsulatum* (Bauman and Chick, 1973; Butler *et al.*, 1988), and *Coccidiodes immitis* (McKenney *et al.*, 1948; CDC/NIH, 1988; Rapley and Long, 1974; Kruse *et al.*, 1967). These organisms are important because they may cause chronic or acute granulomatous or suppurative respiratory tract and bone lesions that resemble tuberculosis. *Nocardia* is partially acid-fast, so lesions can be confused with tuberculosis by both gross and microscopic examination (Jonas and Wyand, 1966; Al-Doory *et al.*, 1969; Boncyk *et al.*, 1975; McClure *et al.*, 1976). *Cryptoccus neoformans* usually causes meningitis in humans but may cause granulomatous pulmonary and cerebral lesions in primate animals (Garner *et al.*, 1969; Takos and Elton, 1953; Linares and Daker, 1972). Mucormycosis, caused by *Mucor* spp., tends to be an opportunistic infection with invasion of the nervous system and other viscera, but may also cause localized infection (Gisler and Pitcock, 1962; Hessler *et al.*, 1967).

Superficial mycotic infections of all kinds have also been found in nonhuman primates. *Candida albicans* may cause localized thrush on oral or genital mucosa of infants or a disseminated disease in immunocompromised animals (Henrickson, 1984; Wikse *et al.*, 1970; Kerber *et al.*, 1968). *Dermatophilus*

spp. cause dermatitis with alopecia, which may progress to papillomatous encrustations, with a high relapse rate suggesting that organisms may persist on pelage and continue to be a hazard to handlers after apparently successful treatment (Kaplan, 1976; Fox *et al.*, 1984). The true dermatomycoses, commonly referred to as ringworm and athlete's foot, are caused by *Microsporum* and *Tricophyton* spp. in both human and nonhuman primates (T-W-Fiennes, 1967; Gugnani, 1971; Taylor *et al.*, 1973; Fox *et al.*, 1984; MacKenzie, 1961). Dermatophilosis (cutaneous streptothricosis) in owl monkeys (*A. trivirgatus*) has been reported (Fox *et al.*, 1973).

Prevention: Biosafety Level 2 precautions are recommended for preventing the spread of mycotic diseases from animals to contact workers (CDC/NIH, 1988).

IX. PARASITIC DISEASES

A. Helminths

The helminths include Acanthocephala, the thorny or spiny-headed worms; Nemathelminthes or nematodes, the roundworms; and Platyhelminthes, the flatworms.

1. Acanthocephalans

Human infection with acanthocephalans has not been reported, and the potential for infection is minimal. However, they are highly pathogenic to all nonhuman primates and are found in both New and Old World species (Middleton, 1966; Moore, 1970).

Prevention: The intermediate hosts for acanthocephalans are cockroaches (Whitney, 1976). They are included in this discussion to emphasize the need for vermin control in primate animal housing.

2. Nematodes

The nematodes, round or thread-like worms, include many genera of endoparasites that are pathogens of both human and nonhuman primates.

a. *STRONGYLOIDIASIS.* Strongyloidiasis, caused by *Strongyloides stercoralis*, is an important and significant human parasitic disease because it is difficult to diagnose and has the potential to cause serious or even lethal diseases in immunosuppressed individuals, especially those on steroids (Neva, 1986). The *Strongyloides* spp. of nonhuman primates are very common and equally important parasites. Transmission of both the human and primate strongyle species from monkeys to man has been demonstrated (Hira and Patel, 1980; Whitney, 1976).

Two important differences exist between the life cycle of *Strongyloides* and that of other intestinal nematodes that enhance the ability of these parasites to be transmitted and to survive: (1) larvae, rather than eggs, are passed in the feces, and (2) they are capable of producing internal autoinfection. The rate at which strongyles pass through this life cycle is significant. Rhabditiform larvae appear in the feces within 1 week after infective larvae are ingested so the autoinfection route has also been established within this time; those that pass in feces can mature and reproduce sexually to produce more than 20 times their original numbers in infective larvae in as few as 5 days (Whitney, 1976).

Diagnosis: Demonstration of larvae in a direct fecal smear is the most simple diagnostic method. However, larval excretion is often sporadic and scanty so standard concentration techniques such as the formalin–ether method may fail to demonstrate suspected infections, and the Baerman concentration method (Neva, 1986), which examines 20–50 g of stool, may be necessary.

Several negative stool examinations may not conclusively rule out low level *Strongyloides* infections, so serodiagnostic tests have been developed using larval antigens in enzyme-linked immunosorbent assays (ELISA) and immediate hypersensitivity skin tests. The ELISA test may prove to be valuable for evaluating efficacy of treatment because titers declined or disappeared in most of treated patients in a human study (Neva, 1986).

Prevention: Biosafety Level 2 is recommended for housing research animals with strongyles, but special attention to animal husbandry routines is essential. The short life cycle of strongyles mandates daily removal of fecal waste from animal housing to prevent increasing infection by the rhabditiform larvae that become infective filariform larvae within 1 to 2 days. Animals kept on dirt floors or in bedding are likely to have massive infections because of the ability of this parasite to multiply rapidly in a short-term, free-living phase.

Intensive treatment in conjunction with a strict sanitation program is the only way to diminish infection with *Strongyloides*. Even in human patients using excellent hygienic practices, complete eradication is almost impossible because even a few residual parasites can maintain infection via the internal autoinfection route (Neva, 1986). Protection of personnel in an animal facility requires strict adherence to the requirements of wearing gloves, masks, and protective clothing to prevent fecal material from contacting skin or mucous membranes during husbandry routines or research handling of animals.

b. *ENTEROBIUS.* Almost all primates have their own host-specific species of pinworm, and *Enterobius vermicularis*, the human pinworm, is prevalent in chimpanzees and is occasionally seen in other captive primate animals (Whitney, 1976). They are often considered unimportant commensals, but a heavy infestation can produce intestinal obstruction in infants, and scratching can produce considerable trauma with subsequent local infection. Infective ova of these parasites are deposited on the perianal skin, rather than within the gut, where they are mixed into the feces. Deposition of ova on the perianal and

perineal skin by adult female worms is accompanied by a pricking or itching sensation which stimulates the host to grab or scratch the area, thereby spreading the ova from hand to mouth, for reinfection, from hand to hand to other animals in close proximity, or to environmental surfaces. These ova, which are resistant to drying and remain viable for 3 weeks, become widespread in the air and dust so transmission to people who enter a room where there are infected animals is possible.

Treatment and control: Biosafety Level 2 is recommended with special attention to husbandry. In human patients, treatment is usually not indicated because the parasitic relationship is usually not harmful, prevalence is high, and reinfestation is probable. Complete eradication of pinworms from a primate research facility is not a reasonable goal, but levels of infestation can be reduced by appropriate vermifuge treatment of animals and frequent cage washing.

c. *OESOPHAGOSTOMUM.* More than half of newly imported Old World primates shed the hookworm-like eggs of the "nodular worm," *Oesophagostomum apiostomum*, which also resembles hookworms in the adult phase. Transmission is fecal–oral by ingestion of infective larvae, which hatch in the feces within 48 hr. After ingestion, these pass directly into the mucosa of the colon and induce the rapid development of large, firm, often black, encapsulated nodules. In 5–8 days these nodules rupture, and the worms escape into the lumen of the intestine and mature. In animals immunized by previous exposure, the worms may remain in the nodules which persist and become caseous or calcified. Heavy infection may cause diarrhea, adhesions, or even death. Aberrant lesions occasionally found in human liver and kidney are called "helminthomas" (Whitney, 1976).

Prevention: Biosafety Level 2 with enteric precautions is recommended.

d. OTHER ROUNDWORMS. *Ascaris* spp. are not likely to spread to people or among animals held under sanitary laboratory conditions because the ova require 4 to 5 weeks after passage in feces to reach the infective stage. However, the zoonotic capability of *Trichuris trichuria*, the human whipworm, has been demonstrated by experimental transmission of ova from monkeys to man (Horii and Usui, 1985). Therefore, these and other roundworms, including the hookworms (*Necator americanus* and *Ancylostoma duodenale*), *Terniden diminuta*, and *Trichostrongylus* spp., should all be regarded as zoonoses (Fox *et al.*, 1984).

B. Filariae

Adult Filaroidea are long, slender worms that invade tissues and body cavities. Larval forms are transmitted by blood-sucking arthropod intermediate hosts, and all disease manifestations are attributable to the migration of larvae and their development into adults. The chronic stage of one type of human filariasis, caused by *Wuchereria bancrofti* and spread by common culicine mosquitoes, is frequently associated with elephantiasis. Onchocerciasis, a filarial disease transmitted by blackflies, is a common cause of blindness in Africa. Chimpanzees were experimentally infected with this parasite to test the efficacy of ivermectin in treating this disease.

There are no documented cases of transmission from nonhuman primates to people, but these worms are mentioned because epidemiologic studies in endemic areas of Africa and Asia indicate that nonhuman primates are reservoirs of filariae (Dissanaike, 1979; Liat and Wah, 1978), and the possibility of transmission exists wherever infected animals and insect vectors are found together.

Prevention: Biosafety Level 2 practices, containment equipment, and facilities are recommended. Because these nematodes have the potential for transmission from nonhuman primates to people, biting arthropod insect control programs should be emphasized, and screening and other methods of preventing contact of these vectors and nonhuman primates should be instituted where feasible.

C. Cestodes

The cestodes are tapeworms, most of which are not zoonotic because their life cycles require intermediate hosts that are not usually found in research laboratories and animal facilities. However, *Hymenolepsis nana*, the dwarf tapeworm, does not require an intermediate host and could be of importance in primate facilities. It is found in rodents, nonhuman primates, and people throughout the world. Autoinfection can occur from eggs hatching within the intestine and embryos maturing without leaving the original host. Ova that pass in the feces can spread infection if ingested by another host (Whitney, 1976).

Prevention: Biosafety Level 2 conditions with good sanitation and vermin control are essential to prevent spread of this parasite in animal quarters (Whitney, 1976).

D. Trematodes

Nonhuman primates have been found infected with virtually all of the flukes that also cause human infestation (Abbott and Majeed, 1984), but the requirement of an intermediate host, which is usually a snail or other aquatic invertebrate, precludes natural transmission in the laboratory. Laboratory-associated infections with *Schistosoma* spp. and *Fasciola* spp. have been reported, but none were associated with laboratory animals.

Schistosomiasis, which is caused by blood flukes, is an important human disease. Natural infection of nonhuman primates with *Schistosoma mansoni* and *S. hematobium* in Africa (Else *et al.*, 1982; Fuller *et al.*, 1979) and *S. incognitum* in India (Ahluwalia, 1972) demonstrates the zoonotic potential of these parasites and suggests that nonhuman primates may be important reservoirs of these human diseases (Durfee, 1971). By

virtue of this susceptibility, nonhuman primates have served as animal models for this human disease (Goldsmith and Kean, 1966).

Prevention: Biosafety Level 2 conditions are recommended for all primate animals. The only potential source of laboratory infection with schistosomes would be handling the infective cercariae for experimental infection of laboratory animals. These are normally in water and penetrate skin, thus gloves and protective clothing are recommended.

E. Protozoa

The protozoa are unicellular organisms, many of which are important parasites of human and nonhuman primates. These include agents that cause systemic, respiratory, and gastrointestinal disease.

1. Malaria

Malaria is the most important protozoan disease. The global incidence of malaria is considered to be approximately 110 million clinical cases annually with 270 million people being infected (WHO, 1990a). The malignant tertian form is caused by *Plasmodium falciparum*, and quartan disease is caused by *P. vivax, P. malariae, P. ovale, P. brazilianum*, and *P. knowlesi*. Both the quartan and tertian forms occur in nonhuman primates, and malaria has been shown to be a true zoonosis, both in nature and in the laboratory (Whitney, 1976; Bennett and Warren, 1985; Collins *et al.*, 1973; Kalra, 1980). *P. cynomolgi* infection of *M. fascicularis* and *P. brasilianum* infection of New World monkeys have been transmitted to man by mosquitoes. *P. knowlesi* infection of monkeys has been found as a natural infection in man, as have malarial parasites of African monkeys. Accidental laboratory infections as well as human volunteer studies have proven that *P. cynomolgi* can be transmitted from nonhuman primates to humans via infected mosquitoes (CDC/NIH, 1988).

Prevention: Biosafety Level 2 conditions with special emphasis on insect control are recommended. Universal precautions should be observed when handling animals that may have infectious particles in their blood.

2. Toxoplasmosis

Toxoplasmosis caused by *Toxoplasma gondii* is widespread in humans and lower animals (Fox *et al.*, 1984), but reported cases of infection of nonhuman primates are few. However, baboons (*Papio* spp.), chimpanzees, and *M. arctoides* have been experimentally infected, and laboratory-associated infections have been reported (CDC/NIH, 1988).

Prevention: In the general human population, transmission of *Toxoplasma* is transplacental, by ingestion of undercooked meat, or by exposure to oocysts. Oocysts are common in soil contaminated by cat feces. In the laboratory setting, fecal contamination would be the potential route of transmission, thus Biosafety Level 2 is recommended.

3. Trypanosomiasis

The diseases caused by protozoa of the genus *Trypanosoma* are characterized by fever, lymphadenopathy, localized edema, and frequent progression to meningoencephalitis with convulsions and death. Chagas disease, caused by the hemoflagellate *Trypanosoma cruzi* and transmitted to humans by blood-sucking triatomid bugs, exists only in the Americas, where it is estimated that the overall prevalence reaches 16 million cases (WHO, 1990c). The *Trypanosoma* spp. that cause African sleeping sickness are carried by the tsetse fly, a member of the *Chrysops* genus that includes deer flies.

Prevention: The insect vectors of Chagas disease are found in the southwestern United States, where many nonhuman primates are held in outdoor facilities and there are about 80 North American species of *Chrysops*. Therefore, Biosafety Level 2 conditions with special attention to vector control are recommended.

4. Leishmaniasis

Leishmaniasis, caused by the protozoan flagellate *Leishmania* spp., is spread by sandflies of the genus *Phlebotomus*. Human infection with *L. tropica* produces a nodular cutaneous lesion followed by local ulceration; it is self-healing over a period of weeks to months. Recovery is associated with immunity to infection. *L. donovani* produces kalaazar, a chronic visceral infection in humans. Both are found in monkeys, and *M. mulatta* have been experimentally infected with *L. tropica* to study the humoral immune response (Wolf, 1976). Laboratory-associated infections with these organisms have been reported (CDC/NIH, 1988).

Prevention: Biosafety Level 2 with special attention to vector control is recommended. Infective stages of all of these bloodborne parasites may be present in blood, feces, lesion exudates, and infected arthropods. Depending on the parasite, accidental parenteral inoculation, transmission by arthropod vectors, skin penetration, and ingestion are the primary laboratory hazards. It is almost impossible to prevent all mosquitoes and other arthropods from gaining access to human and animal housing, and chemical insecticides should not be used in animal quarters. However, electrified insect traps with ultraviolet light attraction, advantageously placed near doors in animal rooms, can minimize the risk of vectors transmitting bloodborne parasites.

5. Pneumocystis

Pneumocystis carinii appears to be cosmopolitan; it infects many domestic and farm animals as well as nonhuman primates and humans (McClure and Keeling, 1971; Poelma, 1975). It was known to cause pneumonia epidemics in premature or debilitated infants, but came into the limelight as one of the major

opportunistic infections in patients with AIDS (Durack, 1981). It has occasionally been observed in association with respiratory disease in macaques and may cause pneumonia in animals that are severely debilitated or immunosuppressed (Henrickson, 1984).

Traditionally, the definitive diagnosis of *P. carinii* infection requires demonstration of the organism in the lung or lower respiratory tract by biopsy, but serologic diagnosis by antigen detection is also used (Pifer *et al.*, 1978).

Prevention: Biosafety Level 2 is recommended, and immunocompromised individuals should not work with animals known to be infected with *P. carinii*.

6. Amebiasis

Entamoeba histolytica produces dysentery in both human and nonhuman primates (Haq *et al.*, 1985), but in monkeys and apes an asymptomatic state may exist (Whitney, 1976). Animals with this type of infection can be more dangerous than those with overt symptoms. Although bloody dysentery causes concern, formed stools do not. The trophozoite forms found only in soft stools are fragile, but the infective cysts that are found in normal feces are resistant to drying and chemical actions. These cysts are readily transmitted in or by food, water, insects, and fomites and pose a real hazard to other animals and people in contact with them (Remfry, 1978).

Prevention: Biosafety Level 2 with particular attention to enteric precautions and good hygiene is recommended.

7. Balantidiasis

Balantidium coli, a relatively large ciliate that is common in many primates, especially chimpanzees, is spread by cystic forms via the fecal–oral route. It may appear as a normal commensal in animals, and no cases of zoonotic transmission have been reported, but it causes severe dysentery in human patients (Whitney, 1976).

Prevention: Biosafety Level 2, with special attention to enteric precautions, is recommended.

8. Enteric Flagellate Infections

Trichomonas spp. and *Giardia* spp. occur in large numbers in the intestinal tracts of nonhuman primates (Whitney, 1976). Studies of *G. lamblia* among children of preschool age showed that hand-to-mouth transmission by fecal contamination of fingers, toys, and the environment is the major source of infection in this setting. Infected children served as reservoirs of endemic infection from which siblings, parents, day-care workers, and their friends are infected (Pickering *et al.*, 1984).

Prevention: There is no known zoonotic transmission, but the analogy between nursery school and primate housing facilities is apparent, and the preventive measures of Biosafety Level 2 and good hygiene are applicable to both.

9. Cryptosporidiosis

Cryptosporidiosis spp. are pathogenic agents of diarrhea in both immunologically intact and immunosuppressed humans and nonhuman primates. A reproducible experimental model for studying this disease has been developed in *M. nemestrina* (Miller *et al.*, 1990). The disease is normally an acute, self-limited diarrhea, but it has severe implications for immunocompromised individuals and has become prominent as an opportunistic infection of AIDS patients (Wormser, 1985).

Prevention: Biosafety Level 2 is recommended. Diseases spread by the fecal–oral route can only be contained by good husbandry and personal hygiene.

F. Lice and Mites

The ectoparasites most commonly found on nonhuman primates are lice and mites. These blood-sucking and biting arthropods are not host specific and will move from one warm-blooded animal to another. Both are known to serve as vectors of human rickettsial diseases, e.g., epidemic typhus fever, which is louse-borne, and scrub typhus, which is spread by lice. Although there is no documentation that such diseases have been transmitted from infected monkeys to humans, mites from nonhuman primates were thought to be responsible for dermatitis in human contacts, e.g., pediculosis from spider monkeys (Ronald and Wagner, 1973) and sarcoptic mange from macaques (Smiley and O'Connor, 1980).

The lung mite, *Pneumonyssus simicola*, causes pulmonary acariasis in monkeys and is very common in macaques. This parasite is not thought to be zoonotic, but is noteworthy because the small cystic lesions of this infestation, found throughout the lung parenchyma and particularly on the surface, are often numerous and may be confused with those caused by tuberculosis. Although the names are similar, it is not to be confused with *P. carinii*, the protozoan parasite that may cause severe pneumonia.

Prevention: Biosafety Level 2 procedures are recommended for preventing the possible spread of lice and mites from animals to contact workers.

X. ALLERGIC DISEASES

Allergy to animal dander is well known and has been recognized not only in pet owners but also in laboratory animal workers. Allergic sensitivity resulting in asthmatic respiratory disease from working with research animals, including nonhuman primates, has been reported (Lutsky and Toshner, 1978), but it is not common (Lynch and Burrell, 1982; Petry *et al.*, 1985).

Allergy to the powder in disposable latex gloves is not limited to primate facilities, but the Biosafety Level 2 requirements for all nonhuman primate-associated work increase the probability of having sensitized people who may develop contact dermatitis with varying degrees of erythema, endema, and vasiculation.

Prevention: The protective clothing, especially masks, in Biosafety Level 2 precautions may lessen exposure to animal dander, but individuals with allergic reactions that cannot be controlled by antihistamines should not work around animals to which they are sensitized. The allergic dermatitis resulting from an essential primary biosafety barrier puts affected workers at greater risk for exposure to many organisms, so nonallergenic gloves should be provided for all who need them.

XI. PHYSICAL INJURY

Physical injury to workers inflicted by nonhuman primates was, and probably still is, more widespread than generally known. Veterinarians, investigators, technicians, and others have been victims of assaults, bites, and scratches by nonhuman primates, both large and small. Several brutal attacks by uncontrollable chimpanzees, both caged and escaped, have caused trauma, disfigurement, and loss of digit and hand functions of workers. Bites, especially by monkeys with long, sharp canine teeth, have caused much physical injury over the years. Most penetrating bite wounds result in severe infection if aggressive antibiotic therapy is not instituted immediately. And, of course, bites and scratches by macaques constitute *H. simiae* exposures.

Floor surfaces (see Part A), which often times are wet and slippery in facilities housing nonhuman primates, contribute to slips and falls by workers. These accidents sometimes result in sprains, pulled muscles, back injuries, bruises, concussions, and other physical harm.

Feet/toes and hands/fingers are sometimes crushed when heavy nonhuman primate cages and equipment are moved. Back injuries abound when these, feed, and other items are improperly lifted and moved in daily routines. Cage washers, steam and hot water, all common to nonhuman primate work, can cause severe burns to workers. Inadvertently mixing certain detergents or germicides with sodium hypochlorite (bleach), commonly used in nonhuman primate facilities, and the subsequent emission of toxic chlorine gas have caused severe respiratory system damage to workers.

Workers must be made aware and constantly reminded of the physical dangers of the particular species of nonhuman primates to which they are exposed and of the environment in which they work. Emergency supplies/equipment and standard operating procedures covering worst-case scenarios should be in effect.

PART C. MODEL OCCUPATIONAL HEALTH PROGRAM FOR PERSONS WORKING WITH NONHUMAN PRIMATES

I. INTRODUCTION

Occupational health programs are mandatory for personnel who work with nonhuman primates and for others who have contact with them. This includes contact with both living and dead animals, their viable tissues, blood and body fluids, waste, or living quarters.

This model program is offered as an example for institutional health programs, which should include all of these considerations. It was developed from programs already in effect at several institutions.

II. PARTICIPANTS

Persons who should be enrolled in an occupational health program include all those who handle animals and are involved in the direct care of the animals or their living quarters on a regular basis (usually one or more times per week) or those individuals who have direct contact with animals (living or dead), their viable tissues, blood and body fluids, or wastes on a regular basis (usually one or more times per week.) These individuals are said to have "substantial animal contact." "Occasional" animal workers have only sporadic or episodic exposure but nevertheless should be included in the program (see Part A).

Enrollment should occur prior to exposure of a participant to animals and products, and voluntary participation should be a condition of employment. Individuals who have been identified for program participation and who refuse to do so should be required to sign a statement documenting their refusal. This refusal to participate may be grounds for refusal of authorization to work with nonhuman primates.

Participants in an occupational health program are usually categorized according to the type of animal contact to which they are exposed and the necessary health services components.

III. COMPONENTS

A. History and Physical Examinations

A medical history and preemployment physical examination should be completed prior to allowing anyone to work with nonhuman primates. It is essentially a specific job fitness-for-duty evaluation. Persons with evidence of diseases transmissible to animals (e.g., tuberculosis), immune deficiency, or other

medical conditions such as allergies that may contradict work with nonhuman primates should be discouraged, or prohibited, from working with these animals.

Thorough medical histories taken by trained health professionals should explore past employment experiences regarding time and attendance, chemical dependencies, job fitness, back injuries, accident proneness, and other factors relating both physical and mental health to job proficiency.

The physical examination should be complete and thorough. Other medical services at this time include chest X-ray, complete blood count and differential, blood chemistry profile, urinalysis, serum sample for storage, and tuberculin skin test. Necessary immunizations may also begin at this time.

Periodic physical examinations should be conducted when warranted, usually annual, and should be customized as appropriate for the circumstances.

Special physical examinations may be necessary after nonroutine exposure to a hazardous situation, e.g., bite by a macaque. These examinations should be limited to procedures necessary for monitoring the patient for the health hazard involved.

B. Serum Storage

A 5-ml serum sample for storage should be obtained from all participants and stored in two equal aliquots at $-20°$ C. These samples may be taken upon enrollment, on physical examination, for an illness that may be job related (at the discretion of a physician), and at termination of employment or work assignment with nonhuman primates, if possible. Serum samples should be stored for at least 1 year after the participant has left the program. Some institutions keep samples indefinitely. Serum banking is expensive, and each institution should determine its particular needs in light of economic feasibility.

C. Tuberculosis Screening

All individuals anticipating contact with nonhuman primates should have negative tuberculin skin test results or otherwise have demonstrated that they are noninfectious before having that contact. Tuberculin skin testing should be administered to individuals without prior history of a positive test (10 mm or greater induration at 48 hr) as a normal part of the preemployment physical examination and every 6 months for people with nonhuman primate contact.

Chest X-rays should be required in the initial evaluation of all who have a positive skin test reaction for the first time. They are recommended for individuals with a history of positive reactions (some may be due to prior BCG administration) and those who have had no chest X-ray evaluation during the previous 12 months.

Employees working with nonhuman primates who, on initial examination or subsequent testing, are found to be tuberculin reactors should have a chest X-ray at the time of the first significant reaction, annually thereafter, and when clinically indicated. They should have thorough medical evaluations at each of these times and prophylactic therapy when indicated. Persons with nonhuman primate contact who are determined to be tuberculin convertors should be prohibited from any contact with nonhuman primates until they have received appropriate medical evaluation and/or treatment (see Part B).

D. Immunizations

The first line of defense for personnel exposed to nonhuman primates with infectious agents is immunization, if available. The immune status of all people who are to come in contact with infected animals or their biological specimens should be evaluated prior to the introduction of a new study agent. Those who are not already immune should be vaccinated if a vaccine is available. If active immunization is not possible, passive immunization with ISG may be given a short time before nonhuman primates are infected with certain agents.

1. Tetanus Prophylaxis

Immunization with tetanus toxoid should be in accordance with recommendations of the Public Health Service Immunization Practices Advisory Committee (ACIP) of the CDC. Boosters should be administered every 10 years or as needed (see Part B).

2. Rubeola (Measles) Prophylaxis

Measles is the most frequently reported viral disease of nonhuman primates. Because of the potential personal and public health consequences associated with rubeola infection, all individuals working with nonhuman primates should have documented proof of immunity or should be vaccinated (see Part B).

3. Rabies Prophylaxis

Rabies immunization should be offered to individuals working with nonhuman primates in quarantine (see Part B).

4. Hepatitis Prophylaxis

If chimpanzees, African green monkeys, owl monkeys, marmosets, tamarins, or nonhuman primates experimentally infected with hepatitis viruses are cared for or used at an institution, a major component of the model occupational health program should cover hepatitis (see Part B).

5. Other

a. SMALLPOX. If recombinant DNA vaccine studies are conducted in nonhuman primates, workers must be vaccinated

for smallpox. The CDC is the only source for this vaccine (see Part B).

b. RUBELLA. To prevent people from transmitting this virus to nonhuman primates, workers should be immune to rubella. This is usually accomplished by administration of the MMR (measles–mumps–rubella) vaccine.

E. *Herpesvirus simiae*

If macaque monkeys are cared for or used at an institution, a major component of the model occupational health program should be about *H. simiae* (see Part B).

F. *Herpesvirus saimiri*

If squirrel monkeys (*Saimiri* spp.) are cared for or used at an institution, a component of the model occupational health program should be about *H. saimiri* (see Part B).

IV. RECORDS

A centralized records system is recommended for all participants in the occupational health program. Usually the responsibility for maintaining the record system is delegated to a university department, the institutional health care services, the office of animal care and use, or some similar entity with one or more individuals directly responsible. It is of utmost importance that appropriate monitoring and scheduling of various components are timely and that documentation is thorough. Procedures for monitoring and detecting occupationally caused illness and injury should be in effect. Records should be kept, and maintained permanently, of work assignments, exposures to hazardous agents, injuries (especially animal bites and scratches), and unusual illnesses. A computerized record system is essential for most institutions.

V. EDUCATIONAL PROGRAM

An ongoing educational program for all persons working with nonhuman primates is a vital component of an occupational health program (see Part A).

VI. HEALTH SERVICES/PERSONNEL

Health-care facilities and services available for all persons working with nonhuman primates vary in size and nature from fully staffed on-site hospitals or clinics to on-call coverage by a private provider in remote locations and for certain small programs. Some moderate-sized facilities may have a small clinic area staffed by a nurse. It is advisable that the physician in attendance be briefed in detail well before emergency situations regarding the health hazards to people working with nonhuman primates and that previously established channels of communication function effectively. A qualified occupational health professional should review the occupational health program and records on a regular basis.

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