

CRITICAL REVIEWS IN
TROPICAL MEDICINE
Volume 1

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CRITICAL REVIEWS IN TROPICAL MEDICINE

Volume 1

Edited by

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Preface

Numerous economic, sociocultural, and health problems continue to impede the optimal progress of many millions of people in the developing countries in tropical and other geographic regions of the world. Thus, tropical medicine has many aspects including parasitology, bacteriology, and virology, environmental sanitation and hygiene, nutrition, pharmacology, immunology, agriculture, economics, political science, anthropology, sociology, and behavioral sciences. Like the mythical Proteus, the individual dealing with tropical medicine must assume many roles.

There is a growing recognition of the unique problems of the tropical countries. This has led to concerted efforts by many international agencies to attempt to obtain new tools to control many of the tropical diseases that have defied previous attempts at large-scale control. The involvement of the world's leading scientists and institutions as well as the best talents and resources of the developing countries themselves has inspired considerable research in tropical medicine with an inevitable exponential growth in publications. The new series *Critical Reviews in Tropical Medicine* is being launched to provide topical state-of-the-art critiques of selected subjects in this burgeoning field. Authored by active investigators in their chosen topics, these reviews should be useful for all health professionals, social scientists, and administrators involved in planning interventions, both preventive and therapeutic, in developing regions of the world. Contributions included in Volume 1 span parasitology, infectious disease, immunology, gastroenterology, liver disease, and nutrition. The very variety of subjects discussed in this volume emphasizes the widening horizons of tropical medicine. Future volumes will maintain a balance among the different facets of tropical medicine.

R. K. Chandra, M.D., F.R.C.P.(C)

St. John's, Newfoundland

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Priorities for Medicine and Health in the Tropics

C. E. GORDON SMITH

1. INTRODUCTION

The problems of health and disease in tropical countries differ from those in the temperate zones in several important respects. It is by no means a question only of climate, nor of the parasitic and vector-borne diseases which are largely confined to the tropics and subtropics because their causative parasites require high ambient temperatures for development or because their vertebrate and/or invertebrate hosts are restricted to warm climates. Equally important are the social, nutritional, educational, and environmental factors characteristic of the poverty—both rural and urban—that afflicts the majority of the peoples of most tropical developing countries—poverty not only of income but also of public services and resources, and increasing in many areas because of galloping population growth in the face of slow and often faltering economic growth. In such circumstances, existing knowledge capable of improving health and controlling diseases cannot be effectively utilized, and further advances in knowledge from research will be equally ineffective unless they can be interpreted into economically feasible and socially, culturally, politically, and ecologically acceptable programs of action.

2. THE PRESENT STATE OF HEALTH AND MEDICINE IN THE TROPICS

The high mortality in infancy and early childhood that characterizes the poorer populations of tropical countries is largely attributable to lethal combina-

tions of diarrheal and respiratory infections with undernutrition and, in many areas (especially Africa south of the Sahara), with malaria. These together give infantile mortality rates as high as 150 or even 200 per 1000 and child death rates (aged 1–4) of around 20 per 1000 in the poorer developing countries (World Bank, 1979). Some infections that are relatively mild in industrialized countries are much more clinically severe in populations immunodepressed by undernutrition or by certain concurrent infections such as malaria. The World Health Organization has estimated that diphtheria, pertussis, tetanus, measles, poliomyelitis, and tuberculosis together cause some 5 million deaths annually in developing countries and that, in 1970, less than 10% of the 80 million children born in these countries received immunization.

The mortality rates in adults are much less dramatic, but morbidity rates are very high and largely attributable to infections. Whereas viral and most bacterial infections generally cause short-term illnesses, many endemic parasitic infections cause chronic disease that develops slowly with only slight apparent effects on the individual over periods of years. But because whole populations may be infected by several species of parasite simultaneously, the disability and misery caused by these infections are often greatly underestimated. In certain helminth infections (notably filariasis), the frequency and intensity of infection determine the severity of disease so that an environmental change that increases the worm burden of the population may increase morbidity without much change in the proportion of people infected.

The distributions, incidences, and prevalences of parasitic and vector-borne diseases depend on ecological factors, particularly population densities (and population changes) of the vertebrate and invertebrate hosts of the infections and the degree of contact of the human population with them (Smith, 1964). All of these factors are influenced by human behavior (including travel and migration), both individual and occupational, and by environmental changes (usually man made): the growth of cities, man-made lakes and smaller water impoundments, irrigated agriculture, deforestation, etc. (Smith, 1975). New and serious infections have been recognized in the Third World during the past decade, for example, Marburg and Ebola fevers (Smith, 1978), and infections have appeared in epidemic form in new areas, e.g., Rift Valley Fever in Egypt (Abdel-Wahab *et al.*, 1978).

Although the incidence of noninfective chronic diseases such as degenerative and malignant diseases is steadily increasing in many tropical countries, such conditions predominantly affect a relatively small affluent sector of their populations. By contrast, hypertension and its complications do not behave as simple “diseases of affluence.” Although absent in a few remote and very poor rural communities, they are generally common in tropical developing countries, and in the West Indies they constitute the leading cause of death in adults (Ashcroft and Desai, 1978).

The following paragraphs briefly review the main factors in health and disease in the tropics.

2.1. Poverty

Estimates suggest that in the world today 800 million people are living in absolute poverty, the great majority of them in the low-income (GNP U.S. \$300 or less per capita) countries of sub-Saharan Africa and South Asia. Moreover, the World Bank (1979) estimates that at the end of the century there may still be between 470 and 710 million absolute poor in the developing countries (13–20% of their population) and that even with very strong national policies for the redistribution of resources, these numbers could be reduced only to 300–350 million; where such favorable policies were achieved, they might, however, contribute to fertility control and further reduce these figures. The figures for absolute poverty must, of course, be much smaller than those for relative poverty, in both income and services.

2.2. Population and Urbanization

Of an estimated total world population of about 4300 million in 1979, about three-quarters live in less developed countries, where 2000 million of the expected increase of 2200 million before the end of the century will live. Between 1975 and 1980, annual growth rates have varied among different regions of the tropics from over 3% per year in middle America and western Africa to 1.8% per year in the Caribbean (United Nations, 1980). Although fertility has declined in some regions and countries, the attainment of stable populations is still distant. Even if the World Bank's projection that fertility in India may reach a replacement level (i.e., average two-child families) by 2020 is realized, the age structure determined by previous fertility levels will give continued population growth (to an estimated 1640 million) until 2150 (Haub, 1980). The consequences of growth rates depend, of course, on the base populations to which they are applied. Thus, Kenya's rate of natural increase (3.9% per year) implies an increase of 15.9 million over the next 20 years, whereas Bangladesh (rate 2.6% per year) is expected to increase by at least 66 million during the same period. India is expected to have 23 million births and 10 million deaths in 1980 alone (Bouvier, 1980). Mortality can be summarized by estimates of life expectancy from birth (World Bank, 1979): between 1960 and 1970, expectancy increased from an average of 42 years to one of 50 years in the low-income countries and from 53 to 60 years in the middle-income developing countries (GNP over U.S. \$300 per capita). Of 50 African countries, 47 have expectancies under 50 years, and in 1985 there are expected to be 34 countries in the world (with 6% of the

world population) with expectancies below this level. Moreover, the mortality decline is slowing in less developed countries and may have reversed in some.

The growth of urban populations has been a major and accelerating trend throughout this century. Between 1960 and 1975, the proportions of the populations of low-income developing countries living in towns and cities increased from 15 to 19%, and that in middle-income countries from 37 to 47%. Between 1950 and 1975, their urban populations increased by some 400 million and are expected to further increase by some 1000 million between 1975 and 2000. In 1950, there was only one Third World city with over 5 million inhabitants; by 2000 it is expected that there will be 40 such cities, 18 of them with over 10 million (World Bank, 1979).

By 2,000 the urban populations in Africa and South Asia are expected to further increase by threefold or more, although, in east and west Africa and in south Asia, rural populations will still outnumber the urban by more than 50%. The growth of urban populations results both from their own growth and from migration from the rural areas—of these, the former appears to be the dominant factor. Migration to towns and cities is probably predominantly motivated by the desire for personal economic gain, and, perhaps surprisingly in view of the environmental, educational, nutritional, and health deprivations that vast numbers of these migrants suffer, a large majority are reported to be satisfied that a gain has been achieved. “Even the pavement-dwellers in Calcutta, who had migrated from rural areas, achieved, on average, higher levels of living than they had previously experienced” (United Nations, 1980). This, of course, serves merely to stress the severity of the rural poverty from whence they came. “The urban poor, as much as their rural counterparts, are therefore trapped in a vicious circle in which low incomes ensure poor education, nutrition and health, which in turn lead to low productivity and incomes” (World Bank, 1979).

In many developing-country cities, more than half the population lives in slums or squatter settlements where between a quarter and a third have no access to a safe water supply or to facilities for the disposal of human excreta. In these areas of urban squalor and overcrowding, the provision of education is poor, and drop-out rates are high. Tuberculosis, diarrheal diseases, and malnutrition are highly prevalent, the latter two being the main causes of a high infantile mortality.

The growth of tropical towns and cities has also aggravated certain mosquito-borne diseases. *Aedes aegypti* is responsible for urban epidemics of yellow fever, chikungunya, and dengue. The emergence since 1953 of dengue hemorrhagic fever as a new and severe disease entity, characterized by fever, shock, hemorrhages, and a high mortality in young children, in large epidemics in cities of south and southeast Asia (Halstead, 1966) and later on Pacific islands was probably at least partly attributable to particularly dense *A. aegypti* populations and high transmission rates (Halstead, 1970; Rosen, 1977). In southern India in the early 1960s, there were large outbreaks of chikungunya because of similar

circumstances (Thiruvengadam *et al.*, 1965). In and around areas of Africa and tropical America where forest yellow fever persists, there is always a risk of spread to urban *A. aegypti* populations and thus of urban epidemics. Apart from these viruses, *A. aegypti* has been shown experimentally to be capable of transmitting some 20 other viruses (Berge, 1975). Urban situations provide abundant blood meals for the largely man-biting *A. aegypti* which breeds readily in water collected from street hydrants and stored for domestic use and also in the increasing urban litter of discarded tin cans and motor tires. Over the past three decades, Bancroftian filariasis, previously largely a rural disease, has become highly prevalent in southern Indian cities as a result of a great increase in the breeding of *Culex pipiens fatigans* in latrines, cesspits, and sewage-polluted waters, all of which increase with the spreading slums lacking facilities for excreta disposal. For example, it was not until the early 1960s that filariasis became a serious problem in Hyderabad and Bangalore (Singh, 1967). Similar urban foci are found on the east African coast, and there are signs of their development on the west African coast.

2.3. Undernutrition and Malnutrition

During the 1970s, growth rates of agricultural production in the developing countries averaged 2.6%, considerably below the target of 4% set by the United Nations for the second development decade. Food production grew slightly faster on average but, even so, barely kept pace with growing populations. Even worse, this modest average performance conceals the fact that more than half of the 69 countries for which consumption figures are available actually suffered a decline in home food production and therefore became increasingly dependent on purchased food imports and on food aid. Even if home production together with imports is sufficient to meet the theoretical requirements of the population of a country, maldistribution usually results in some people getting less than they need. The United Nations (1980) estimates that some 400 million people have calorie intakes sufficient only to support the lowest minimum needs for existence. Although not all of these will have clinical symptoms of malnutrition, they do constitute the population at risk. In the main, they are poor: farmers with access to so little land or to such poor land that they can neither grow enough food for their families nor enough cash crops to exchange for food; landless laborers; casual laborers; and the urban poor. Within the families of the poor, those most conspicuously affected by malnutrition are pregnant and lactating women, infants, and young children. The effects of inadequate diets often interact synergistically with those of infectious diseases: nutrient deficiencies are immunosuppressive, and the infections themselves enhance the metabolic effects of the deficiencies. In addition, illness often reduces appetite, lowering food intakes still further. A study of 35,000 deaths under 5 years of age in Latin America (Puffer and Serrano, 1973) showed that 34% had malnutrition as an

underlying or associated cause, for the most part together with diarrhea or upper respiratory infections.

Obviously, there can be no easy or rapid solution to this large and complex problem. An increase in agricultural productivity is clearly a prerequisite, but this has to be matched by an increase in effective demand for food by the poor and accompanied by an extension of primary health care. These, in turn, will only bring real gains in health if the domestic environment reaches minimal standards of safety—water, sewage, and fuel supplies are in many situations likely to limit the effective utilization of food.

In the main, the nutrition problem is one of undernutrition rather than malnutrition. That is to say, intake of all nutrients and energy are low. In the late 1960s and early 1970s, it was believed that protein deficiency was the main factor in malnutrition; it is now accepted that energy intake is the main limiting factor for both adults and children (Waterlow and Payne, 1975). Obviously, both energy and protein are required, and the dominant factor is lack of enough to eat. If most populations could eat enough of what they already eat, most of their problems of malnutrition would be solved.

Apart from undernutrition or malnutrition, there are more specific nutritional deficiencies, some of which are important in the tropics. Iron deficiency anemia is very common in infants, young children, and women (particularly during pregnancy) throughout the developing countries, prevalences as high as 80% in these groups having been reported (World Health Organization, 1975). Iron absorption from vegetarian diets tends to be poor and aggravated by infections; malaria and hookworm infection further aggravate it by blood loss. Vitamin A deficiency is frequently combined with general malnutrition, affects children, and can cause permanent blindness; it has done so in perhaps as many as 180,000 in the world today (World Health Organization, 1979a). Iodine deficiency, with goiter and cretinism, is still common in many parts of the world including tropical developing countries, particularly in mountainous regions.

Improvements in the pattern of distribution of food, in effective demand for food, and, at the same time, in the quality of the domestic and health environment, attaining levels that insure the effective biological utilization of food, can only be brought about if the objective of improving nutrition is included in the choice, design, and management of agricultural development projects. During the last few years, FAO has been developing and testing techniques for including nutritional criteria in project appraisal procedures (Food and Agriculture Organization, 1979). A basic requirement for the application of these techniques is reliable information about the state of nutrition of population groups likely to be affected by development projects, in particular, of course, about poverty groups. Valverde *et al.* (1981) have described an information system that has been designed to provide both base-line measurements and continuous surveillance of critical risk groups in Costa Rica.

Some communities, and particularly those in countries with declining food

self-sufficiency (which includes most of Africa), will be exposed for a long time to come to a high risk of failure of food supply. In the main, these risks tend to be aggravated by exaggerations of the effects of regular seasonal fluctuations and by unusually bad years resulting either from climate or from a man-made phenomenon such as a rise in world prices. Methods of improving seasonal food security will need to be based on a better understanding of the way in which the cycle of agricultural labor often results in critical periods of high food requirement for the working members of farming families: frequently, these coincide with the rains, and in tropical areas these are just the times when many water- and insect-associated diseases are at their maximum. Thus, poorest health and greatest nutritional stress are often associated with maximum demand for labor (Chambers *et al.*, 1979).

2.4. Drinking Water and Excreta Disposal

The World Bank (1980) estimates that 1500 million people in developing countries (excluding China) lack adequate supplies of safe water and adequate waste disposal facilities. Between 1100 and 1200 million of these live in rural areas (i.e., over 80% of their rural populations), 300 to 400 million in urban areas (i.e., 50–66% of their urban populations). Figures for access to water supplies do not necessarily imply access to reliable ones. For example, in Indian cities, the proportion of the population reported to have access to water supplies increased from 60% in 1970 to 80% in 1975. But in the largest cities, those who had 8–10 hr of service in 1970 had only 2–3 hr daily in 1975. And whenever water supplies are interrupted, their safety is in doubt because of suck-back. The extent to which diarrheal diseases in the tropics are water borne, food borne, or transmitted by more direct fecal–oral routes requires careful laboratory-based epidemiological study. Feachem (1978) has drawn attention to the probable importance of “water-washed” or “water-scarce” transmission, where personal cleanliness and the cleanliness of domestic utensils are important. For such transmission, the quantity, availability, and reliability of water supplies are more important than their quality. Without plentiful and easily accessible water, improvements in cleanliness cannot be expected.

Probably only about a quarter of Third World populations have adequate means for waste disposal, and in congested urban areas, these are commonly no more than latrines, which are usually unsatisfactory and often unacceptable and are often not designed for use by children. Poorly designed, constructed, or sited privies often contaminate nearby shallow wells. The lack of satisfactory water supplies and excreta disposal services or facilities is probably responsible for (or contributes to) many infant deaths and a high proportion of morbidity in adults in tropical developing countries. There may well be 30,000 deaths a day attributable to inadequate water supplies (*Lancet*, 1981). The poor, particularly women and children, suffer most from the deficiencies, and the productive potential of

poor households is impaired by them. In rural areas it is often necessary to walk 1–5 miles to obtain acceptable water, or in urban areas to wait for hours in queues at public standpipes. If pure water is hard to obtain, there is a strong temptation to use more convenient sources which may be contaminated.

The provision of safe drinking water can alone be expected to have only a modest effect on this burden of disease except in arid areas where excreta dry very rapidly and organisms in them die quickly. In other tropical areas, the safe disposal of human excreta is essential to prevent contamination of water, food, and soil and thus the transmission of viral, bacterial, and parasitic diseases. The transmission of schistosomiasis can be as frequent in urban as in rural areas if the sanitary conditions are similar (Costa *et al.*, 1980). Inadequately designed and maintained sanitary facilities enhance the transmission of Bancroftian filariasis. In Pondicherry, drains and cesspits made the greatest contribution to *C. pipiens fatigans* breeding, and the numbers of mosquitoes emerging were estimated as 173,000 per day in November, increasing with rainfall to 9 million per day in January (Menon and Rajagopalan, 1980).

The effective use and maintenance of water and excreta disposal facilities require health education appropriate to local customs, beliefs, and conditions, and those who aim to provide “appropriate technology” in tropical villages must first make the effort to comprehend village technology, recognizing that it has evolved by perhaps centuries of trial and error and by the elimination of “inappropriate technologies.” A stage-by-stage (“incremental”) approach to the provision of sanitation in poor urban communities is described by Mara and Feachem (1980).

In 1980, the United National General Assembly launched the International Drinking Water Supply and Sanitation Decade with the overall objective of providing for 900 million urban and 1600 million rural people between 1975 and 1990. Specific targets established by each country will take into account the costs, resources, and competing needs of other sectors. The World Bank estimates that the cost of full achievement of the objective might rise to \$30 billion a year and that even so, in 1990, there will still be 1500 million people unserved. Although short of trained staff, urban services may be capable of being strengthened relatively rapidly provided that there is sufficient political and administrative will. Rural developments are likely to be inhibited mainly by “extreme shortage of staff trained to promote rural services and to organize communities for the purpose” (World Bank, 1980).

2.5. The Plasticity of Pathogens and Vectors

Most of the pathogens that infect man and their invertebrate hosts are rapidly multiplying and evolving entities. The successive antigenic changes in the influenza viruses under the selective pressure of immunity in man and other

species lead to new variants at frequent intervals and to major variants causing large epidemics every few years. Thus, the antigenic content of vaccines must be frequently adjusted and depends on worldwide surveillance organized by WHO for detection of current and new strains. Smallpox could be eradicated because the virus was similar everywhere and because variants sufficiently different to infect vaccinated individuals did not emerge. However, closely related viruses (monkey pox) continue to be isolated, and when the level of herd immunity wanes, now that vaccination has ceased, these may prove capable of adaptation to man. Thus, continued vigilance is essential and will be difficult to maintain as fear of smallpox disappears. For the moment, no other virus disease is a serious candidate for eradication, but viruses that are relatively invariant and infect only man will become possibilities if a highly stable and effective vaccine (and sufficient funds to use it) become available worldwide and if the disease itself can be relatively easily diagnosed without major laboratory facilities. Measles, perhaps a possible candidate, does not yet meet either of these criteria in the developing countries.

Of bacteria, there are some that undergo antigenic variation (the *Borellia* species causing relapsing fever) and some groups with many species (e.g., *Salmonella*), but the most important change is towards resistance to antibiotics and chemotherapy in the face of widespread and often inadequately controlled use of these drugs. This widespread use, even by quite poor people in poor countries, must be discouraged by controlling availability; in the Third World, its individual benefit depends largely on chance, and disadvantage to the community is a certainty. To take one example, Echeverria *et al.* (1978) found that 76% of enterotoxigenic *Escherichia coli* isolates from the Philippines, Korea, Taiwan, and Indonesia were resistant to one or more antibiotics, 44% to four or more. Antibiotics were available in the Philippines without prescription, and of 1000 children admitted to hospital with gastroenteritis, 980 had been given antibiotics before admission. Antibiotics are similarly widely used in Indonesia and Taiwan.

The treatment of leprosy has depended for the past 30 years almost entirely on sulfones. Although this treatment has perhaps reached only 20% of those requiring it, and the disease continues to be very widespread, drug resistance is widely distributed and increasing. No satisfactory prophylaxis exists, and there is an urgent need for a cheap alternative to dapsone (Browne, 1980).

Drug resistance is similarly a problem in the treatment of tuberculosis worldwide. With data from a limited number of countries between 1970 and 1973, the World Health Organization (1980b) estimated that tuberculosis accounted for 66% of the deaths from chronic respiratory disease in Asia, 54% in Oceania, and 11% in Africa. The outstanding success of the penicillin campaign against yaws was largely attributable to its not becoming resistant. However, with the exception of the treponemes, where a widespread and prevalent infection is treated on a large scale with an effective drug, resistance tends to appear,

and the more effective drugs there are, the more complex the resistance patterns.

This appears to apply to the protozoa infecting man. Malaria is the only such infection for which effective drugs have been in widespread use over a long period. In recent years resistance to the main chemotherapeutic agents has been an increasing worry in both treatment and prophylaxis. Resistance to pyrimethamine is already widespread in African *Plasmodium falciparum*. Chloroquine resistance is more serious: it began about a decade ago in limited areas of southeast Asia and Central America and has spread steadily to become widespread in most of south and southeast Asia. Its spread in India appears to have been accelerated by migrant labor populations. Chloroquine resistance was not identified in sub-Saharan Africa until 1978 but has now been clearly demonstrated in a few cases and may become established and spread. The alternatives to chloroquine for the treatment of malaria tend to be rather more toxic; some of these are mixtures of drugs, and resistance to some of them is already beginning to appear. Some of the mixtures contain a sulfonamide or sulfone, and their widespread use, particularly for prophylaxis, might contribute to sulfonamide resistance in infections such as cerebrospinal meningitis in its sub-Saharan epidemic belt or to dapsone resistance in leprosy.

There appears as yet to be no evidence of significant drug resistance in helminths, perhaps because of their greater complexity, morphologically, physiologically, and in terms of life cycle, and because as yet no highly effective drug has been in widespread enough use for a long enough time. The only such drug, diethyl carbamazine, kills only a proportion of the adult worms in *Filaria bancrofti* and *Brugia* infections and probably none in onchocerciasis.

The same general rules appear to apply to vectors and insecticides, except that the latter are applied much more indiscriminately. Thus, insecticides used in agriculture find their way into the breeding places of medically important mosquitoes and probably contribute to selecting resistant strains. The malaria vectors represent the largest single medically important resistance problem. Between 1968 and 1980 the number of resistant anopheline species increased from 38 to 51. Most of them are resistant to dieldrin, and 34 of them to DDT. Multiple resistance involving these, carbamates, and one or more organophosphorus insecticides has appeared in ten species (Davidson *et al.*, 1980), among them *Anopheles albimanus* in Central America (Georghiou, 1972) and *A. saccharovi* in Turkey (Ramsdale *et al.*, 1980). The latter caused a recent intractable outbreak of over 160,000 cases of malaria. Malathion is now usually the insecticide chosen when DDT and dieldrin resistance has become a problem. Focal resistance to malathion has already appeared in *A. culicifacies* in parts of India (Herath and Davidson, 1981), and its arrival is feared in Sri Lanka. Resistance to organophosphorus insecticides tends to carry a high degree of cross resistance to other members of the group and even sometimes outside it. Insecticide resistance

is widespread among arthropod species exposed to consistent selection pressure by exposure to insecticides but does not appear to occur so readily in certain species (World Health Organization, 1980a). In the WHO Onchocerciasis Control Programme in the Volta River basin, where, for periods of 5 years or more, there has been regular treatment of whole river systems with the insecticide abate, resistance has appeared only recently (Guillet *et al.*, 1980) and so far only in *Simulium sanctipauli* and *S. soubrense*, which are forest vectors, but not in the main savannah vector, *Simulium damnosum*, which is the target of the control program.

There is a clear need for developing countries to develop a policy for the control and regulation of the use of chemotherapy for the treatment and prophylaxis of infections, although it must be admitted that even very sophisticated countries have been unable to regulate chemotherapy sufficiently to prevent the development of increasing drug resistance in bacteria. The need for agreement between the agricultural and health users of insecticides about their best use to avoid increasing resistance, which affects both sectors, has often been discussed, but little progress has been achieved. With resistance and cross resistance ever increasing, the need for such a policy is also increasing lest chemical ingenuity fail and both sectors be left without the weapons they have come to rely on.

2.6. Ecological and Environmental Changes

Many tropical infections have vectors (e.g., mosquitoes in malaria) or alternate hosts (e.g., snails in schistosomiasis) or are zoonoses—infections of man that are not normally transmitted from man to man but are maintained in another vertebrate species and transmitted to man directly (e.g., rabies), through soil (e.g., anthrax), water (e.g., leptospirosis), food (e.g., salmonellosis), or by blood-sucking arthropods (mosquitoes, ticks, tsetse flies, sandflies, midges, etc.). The risk of infection with schistosomiasis or malaria, for example, depends on the population density and infection rates of the responsible snails or mosquitoes and on the degree of exposure to them, i.e., water-contact in schistosomiasis, exposure out of doors to a mosquito that bites in the evening or at night, the nonuse of mosquito nets against mosquitoes that bite indoors. The risk of infection with zoonoses depends on the population density of the maintenance species and the degree of contact with its excreta or with the vectors that transmit it to man. In either case, the importance of increased population densities of intermediate hosts or vectors is self-evident, and many types of environmental change, often man made, can increase their populations and enhance the incidence of infection.

Water developments are particularly important. For hydroelectric power, irrigation, or urban water supplies, more and more large and small dams are being built throughout the tropics. They are often important sources of

schistosomiasis (Smith, 1975). When, around 1960, the Volta dam in Ghana first filled, aquatic weeds harboring snails were widespread, and schistosomiasis reached epidemic proportions among both migrants to the area and local people. The main sources of schistosomiasis around the Lufira Lake in Zaire were the associated swamps, slow-flowing tributaries, and canals (Ripert *et al.*, 1969). However, the myriad of small dams, often unplanned and built by self-help, may be quantitatively much more important. Lakes with shallow shorelines or where wave action produces sand bars and shallow pools can be important sources of mosquito breeding, especially of *A. gambiae* in Africa. Road making, construction work or neglect of urban drainage can all create shallow pools that breed malaria mosquitoes.

Irrigated agriculture can be a prolific source of snails and schistosomiasis (e.g., the Gezira scheme in Sudan and many areas in Egypt) and of medically important mosquitoes. Throughout its distribution from India to Japan, Japanese encephalitis is transmitted by rice-field-breeding mosquitoes of the *C. vishnui* complex. Food crops encourage dense small mammal populations and intense foci of the infections they carry, e.g., leptospirosis or Argentinian hemorrhagic fever. Food storage (e.g., in granaries) can enhance small mammal populations close to houses and may be a factor in transmitting Lassa fever virus to man from *Mastomys* (Monath, 1975). Deforestation or cattle grazing in forests can intensify zoonoses and increase the risk to man—Kyasanur Forest disease, a tick-borne infection, became epidemic when grazing cattle enormously enhanced the population of a forest tick (Boshell, 1969).

Although, under the influence of the international and bilateral donor organizations, large-scale projects now tend to be planned with some regard to their health consequences, and some financial provision is often available for their assessment and control, little regard is usually paid to the long-term recurrent costs of control and prevention (e.g., of schistosomiasis or malaria). But the myriad of small-scale development activities, especially dams and irrigation, can have an insidious effect on health that is neither recognized nor coped with (Tayo *et al.*, 1980).

New communication systems such as the trans-Saharan or trans-Amazonian highways may also enhance the spread of disease by providing rapid transportation for infected people, vectors, and small mammal hosts of infections and thus the opportunity for their establishment in new areas (Marques, 1979). An outbreak of cattle trypanosomiasis has been attributed to the inadvertent transport of tsetse flies in aircraft (Lovemore and Bax, 1972); the triatomid vectors of Chagas' disease are being transported by road and rail (Schenone, 1971), and vectors of onchocerciasis by road vehicles (Walsh, 1972).

Industrialization, important and increasing in nearly every developing country, is bringing increasing occupational disease problems (World Health Organization, 1972). New industries are developing, some of them with primitive

technologies [e.g., lead poisoning in mushrooming “cottage industries” in motor vehicle batteries (Joshua *et al.*, 1971; Sofoluwe *et al.*, 1971)] and some with new technologies involving potentially dangerous chemicals or dusts. These problems will be neither recognized nor controlled unless there is informed surveillance both of the health of workers and of the processes they are engaged in. Tropical developing countries need to inform employers and employees about occupational hazards (which are not, of course, confined to factories) and about how they can be minimized.

2.7. Major Groups of Diseases

Although the importance of certain groups of diseases is clear, no reliable estimates exist of their incidence or prevalence except in relatively few well-studied areas. Available surveys tell us more about the distribution of investigators than of diseases; data from national sources are fragmentary and biased by their focality. For instance, in Kenya 40% of patients attending a health center came from within a 5-mile radius, 70% from within a 10-mile radius (Fendall, 1965); and in India the proportion of patients attending a dispensary decreased by 50% for each additional half mile they had to travel (World Bank, 1975). Moreover, few if any of the diagnoses from such sources have been confirmed in the laboratory.

In order to overcome this dearth of data, WHO (Cockburn, 1971) asked 112 tropical and subtropical countries to nominate their major health problems. Collated by WHO Region, all regions nominated diarrheal diseases and malaria, and all, except the Eastern Mediterranean, malnutrition. Tuberculosis, leprosy, and sexually transmitted diseases were each nominated by three regions. In a similar assessment of respiratory diseases, WHO (1980b) estimates that between 1970 and 1973 tuberculosis accounted for 60% of the deaths from chronic respiratory diseases in Asia, 54% in Oceania, and 11% in Africa. However, acute respiratory diseases accounted for 13% of all causes of death in developing countries in Asia and for 42% in Africa. In children aged under 15 years in Africa, the mortality from acute respiratory diseases is estimated at 252 per 100,000 (23% of all causes), and for infants under 1 year of age the death rate exceeds 2000 per 100,000 (1 in 50) in some countries. In a study in rural Indonesia of 877 children under 5, there were 3000 episodes of illness in a single year, 45% of them acute respiratory disease, and Indonesian health center data on 6.5 million outpatients in 1977 showed that 44% were suffering from “influenza-like” illnesses.

Sexually transmitted diseases are increasing in prevalence virtually worldwide, and figures as high as 10–20% of the population are reported by WHO (1978a). Penicillin resistance is increasing in gonococci: in Singapore, Malaysia, and Thailand 20–40% of strains are resistant. Female infertility and ectopic pregnancy have increased everywhere in parallel with sexually transmitted dis-

eases. In parts of Sudan, Cameroon, Zaire, and Gabon, 20–40% of women complete their reproductive years without giving birth to a child, and high levels of childlessness have also been reported in east Africa (Catterall, 1981). In Upper Volta, Retel-Laurentan (1967) was able statistically to relate high infertility in villages with gonorrhea imported by the men returning from working away from home, a very common necessity in that part of the world. Childless women carry a serious social stigma in some cultures, increasing the social significance of sexually transmitted disease, particularly gonorrhea.

In 1976, the UNDP/World Bank/WHO Programme for Research and Training in Tropical Diseases was launched as a major effort of research and development to find improved ways of preventing and treating malaria, schistosomiasis, filariasis, trypanosomiasis, leishmaniasis, and leprosy. The strategy and support for research are managed by Scientific Working Groups, and the Research Strengthening Group (RSG) is concerned with relevant training and with strengthening institutions in developing countries so that they can play a full part in collaborating with research programs elsewhere in the world (UNDP/World Bank/WHO, 1979).

Malaria is holo- or hyperendemic in most of sub-Saharan Africa. The World Health Organization (1978a) reported that in India microscopically diagnosed and reported cases had increased from 3.2 million in 1972 to 7.5 million in 1976. *Plasmodium falciparum* resistant to chloroquine was occurring in areas with a population of over 45 million, and some 90 million were at risk; and at least a third of all malaria control programs were facing insecticide resistance. During the past 5 years, this situation has deteriorated in most affected countries. The Special Programme has identified an urgent need for drugs with prophylactic and tissue schizonticidal action, especially for a long-acting (around 3 months) prophylactic drug, and for a single-dose radical cure of *P. vivax* malaria. These drugs should be sufficiently safe and cheap to be administered on a large scale to rural populations. Of new drugs, mefloquine is in clinical trial, and triazines, quinazolines, and a new class of drug developed in China are all being tested. Extensive efforts are also under way to develop a vaccine and to improve immunodiagnosis. A practical vaccine demands the production of antigens in sufficient quantity, their preparation, and their formulation in an acceptable form with, if necessary, an acceptable adjuvant, and satisfactory storage and stability properties. First priority is being given to antigens of blood-stage parasites and socioeconomic research is also in progress (UNDP/World Bank/WHO, 1979).

In schistosomiasis, the Programme gives highest priority to epidemiology and control studies. In the Volta Lake, 2 years of snail control by focal mollusciciding reduced snail density by about 55% and snail infection rates by about 95%. Through treatment of all known infective individuals, the prevalence of the infection in man was reduced by 53%, egg output by 75%, and incidence by 63%. With the advent of praziquantel (a drug effective against all three types of

schistosomiasis), chemotherapy prospects have improved. However, the drug is expensive and likely to remain so because its synthesis is complex. It is concluded that parasite control methodology has now been developed to a point where, if a government were to decide to give high priority to controlling schistosomiasis, a main constraint would be lack of epidemiologically trained personnel to execute and monitor it (UNDP/World Bank/WHO, 1979).

In filariasis, the main priorities are for a safe and practical schedule of treatment for onchocerciasis patients at risk of blindness, for a safe and effective onchocerciasis adulticide, for a practicable schedule of mass treatment to control lymphatic filariasis, and for drugs to control the severe allergic reactions to microfilaricides which are associated with the treatment of all types of filariasis.

The WHO Onchocerciasis Control Programme, which covers the savanna zone of seven West African countries (Benin, Ghana, Ivory Coast, Mali, Niger, Togo, and Upper Volta), started in 1974. By mid-1977, the original area defined had been covered, and in 1978 control was extended in Ivory Coast. The Programme now covers 764,000 km². Control of the blackfly vector is effected by the aerial insecticide treatment of its breeding sites in rivers throughout the area. This control has been highly successful except in areas where blackflies invade from surrounding areas, mainly in the west of the Programme area, where the flies are carried long distances by the prevailing wind (Walsh *et al.*, 1979). Epidemiological assessment of the effectiveness of the Programme has shown that where vector control is established new infections are rare and that new cases of blindness may be being prevented (Thylefors and Tønjum, 1980). However, the Programme was planned for 20 years, as onchocerciasis will not cease to be a problem until the prevalence of infection in the population can be reduced to a level at which new infections will be rare or cease. Because the adult *Onchocerca volvulus* lives for perhaps 10–15 years in the infected individual, prevalence of the infection will fall slowly, and vector control must be maintained. No drug suitable for mass chemotherapy and capable of killing the adult worms is available.

Improved drugs are urgently needed for the treatment of trypanosomiasis, both African and American (Chagas' disease). Experiments on the aerial insecticide control of tsetse flies have encountered the difficulty that the flies travel more than 10 km. Chagas' disease is largely a problem of poor housing, in the cracked walls of which the vector triatomid bugs breed and rest; a trial of housing improvement is in progress in Brazil.

Leishmaniasis, especially kala azar, is an increasing problem with perhaps 400,000 cases annually of all types in America, Africa, and Asia. There is an urgent need for improved treatment (UNDP/World Bank/WHO, 1979).

In leprosy, the priority is for a safe and effective vaccine. Large quantities of the bacillus can be produced in highly susceptible armadillos, and a variety of killed and purified vaccines are under development. Eventually, it should be

possible to produce protective antigens by DNA recombinant techniques. Unless there is a substantial improvement in chemotherapy within 5–10 years, control by chemotherapy is likely to be in severe jeopardy because of sulfone resistance (UNDP/World Bank/WHO, 1979).

More recently, WHO has launched a Diarrhoeal Diseases Control Programme (World Health Organization, 1979b) which will encourage much of the research necessary to back up the International Water Supply and Sanitation Decade. The effectiveness of water supply or sanitation interventions in reducing diarrheal diseases will probably be most influenced by the use made of facilities, especially for excreta disposal. This, in turn, will depend on understanding of the customs, beliefs, and levels of education of user communities. It is much easier to install an excreta disposal system than to ensure its proper use. Any facilities need to be simple in design and easy to install, operate, and maintain. The efforts of the Decade must therefore be closely interwoven with those to develop effective health education and primary health care and will require coordination at all levels from the ministries to the communities served. The Programme is focusing on the pressing needs for trained personnel scarce in the Third World: sociologists and anthropologists trained in relevant aspects of technology, science and medicine; epidemiologists and environmental microbiologists; sanitary engineers with relevant training in the planning and evaluation of programs. There is a wide-ranging need for research.

2.8. Health Services

During the past two decades there has been increasing awareness in developing countries (and in those who aspire to assist them) that the industrialized country model of health care is “inappropriate” or, at any rate, economically impracticable for the vast majority of their populations. In some developing countries, a few large hospitals supplying high technology medicine can consume more than half of the entire health budget. Such hospitals are, of course, in cities and offer little or nothing to rural populations. During the 1960s, emphasis was placed on extending health services more widely through health centers, dispensaries, mobile clinics, and the like. Even these, however, failed to reach more than the populations within reasonable distance, and overall coverage remains poor in most countries.

The World Health Organization now sees the solution in primary health care (World Health Organization, 1978b) defined as “essential health care made universally accessible to individuals and families in the community by means acceptable to them through their full participation and at a cost that the community and country can afford.” The key person is seen as the community health worker who has a brief training and then returns to his/her own community to lead and develop self-help health care (Vaughan, 1980). The following addi-

tional features are to be inherent in this system: attention to political and socioeconomic factors affecting health and development (especially in rural communities), community participation and its control of its own priorities and services, linkage with professional services through the community health worker, and collaboration with and utilization of traditional forms of health care, particularly traditional midwives. There have been a number of successful primary health care projects in various countries under different conditions. However, those that have been successful have, in general, been in fairly remote areas where they posed little threat to government or to the medical profession; they have usually been led by someone with exceptional leadership qualities; they have been small enough not to generate a strong bureaucracy; their hidden costs have been borne, not by the government, but by aid donors, universities, or other nongovernment organizations; and usually they have been initiated by nongovernment or voluntary agencies (Golliday and Koch-Waser, 1977). Can these limited experiments be institutionalized by governments to cover whole countries and be the basis for "Health for All by the Year 2000?" Many developing country governments have committed themselves to this approach, but experience has shown that such developments cannot be financed by redistribution of resources from existing hospital services, and ministries of health will therefore have to acquire a larger proportion of national resources than hitherto.

Although national and multinational aid sources are generally sympathetic and will probably provide capital and short-term assistance for primary health care programs, it is not clear how many countries will be able to sustain the longer-term recurrent costs. Moreover, even remote villagers know, or soon will know, about modern curative medicine, and unless there is a visible and active chain of referral services upwards from the community health worker, his credibility may not last long. He or she needs to be part of an organization of peripheral health service units including dispensaries, health centers, and district medical services. Most countries have administrative and political units with populations of around 100,000–300,000, often called a district, and this is the level at which planning, coordination, implementation, management, and evaluation of health services most sensibly lie. Smaller-scale organizations tend to too great individualism to fit into a larger structure, and national planning often fails to penetrate below "district" level or to take sufficient account of local demographic and ecological (even tribal) circumstances. The "district" is usually the lowest level at which important decisions involving intersectoral interaction can be taken: rural development needs to go hand in hand with the development of rural health services. The "district" is also a good level where nongovernment organizations working "at the periphery" can collaborate with government and where the cooperation of the private and traditional sectors of health care can be enlisted. These are important resources in many countries.

The need, therefore, is for broadly and well-trained health teams at "dis-

trict'' level that can interpret the health needs and priorities of its population, can plan, implement, manage, and evaluate the use of available resources, can formulate credible requests for additional resources, and can collaborate with other sectors in planning and administration.

3. PRIORITIES AND NEGLECTED AREAS

This catalogue of problems and difficulties might suggest that the problems of health and medicine in tropical developing countries are quite insuperable. But despite these formidable difficulties, progress is being made in many countries, several important initiatives towards improvement have been made by the international organizations, and despite their very luke-warm interest in and response to the Brandt Report, the industrialized countries must continue to help. Clearly, only a broad and integrated attack on the many and interlocking facets is likely to yield major improvements. The immediate priority is for the Third World countries themselves to take a greater initiative in developing more effective health policies, reiteratively improved by evaluation and informed refinement, for the better use of their own resources and of those they can obtain through international or bilateral aid programs or can realize by cooperation among themselves. The realization of more appropriate and effective policies demands better understanding of health problems and of what can be done about them at all levels from central government to the individual in the rural village or urban squatter area; and it demands better use of existing and new knowledge, its adaptation to local needs and feasibilities, and effective structures for planning, implementation and management. For all of these, governments need to retain, develop, and effectively harness national scientific expertise.

The Brandt Commission (1980) points out that "only if governments are committed to enabling the poor to benefit from increasing growth can the plea for increased international assistance and cooperation command moral strength." However, it points out elsewhere that

for most developing countries of Africa and Asia, the seeming failure to distribute wealth is a symptom of a deeper distress which many of them (i.e., those with a GNP less than \$250) do not have the resources to tackle. . . . Their total resources, even if they were equally divided, are insufficient to support their populations.

Nevertheless, even the poorest countries could be "richer" if they were able to use their resources more effectively and more appropriately, and better distribution may yield disproportionate advantages. The Brandt Commission (1980) points out that

the countries where birth rates have recently most declined have usually been those which have managed to spread the benefits of development widely. Even very poor areas, such as Kerala in India, have been able to give people new hope for a better life

by involving them in the workings of development, improving their health, raising the status and educational levels of women as well as men, and ensuring adequate food supplies for the poor. Where this has been done, birth rates have tended to fall, while they have remained high in many richer developing countries which have paid less attention to the needs of the many.

Even if "health for all" cannot be achieved (even in the richest and most sophisticated countries) by the year 2000, reasonable access to health care, freedom from hunger, clean drinking water and effective facilities for excreta disposal should be possible for all if the developing countries can achieve more self-reliant attitudes and a more equitable distribution of their resources and if complementary changes can also be brought about in the attitudes of those who must aid them. But time is running out, and these changes need to be made within the next half decade or so.

3.1. Better Understanding: Health Education

There is widespread lip service to the overpowering need for a better public understanding of health and disease and to the reductions in the incidence of a wide range of diseases that could be brought about by changes in individual and communal behavior in the tropics. But relatively little is done: no real investment in expertise or money is made either in creating the capability or in implementation, and what is done is not evaluated. That there is little to quote in the way of results is not therefore surprising. Several of the most important tropical diseases could certainly be reduced in incidence by informed behavior, and in many poor urban or rural tropical communities, little else seems likely to be done in the foreseeable future about some of these diseases. Minimization of water contact by provision of clean and acceptable facilities and by their educated use would reduce exposure to schistosomiasis in many places where, whatever advances are made by research, control schemes are unlikely to be feasible because of the dispersed nature of the foci of infection. The provision and educated use of latrines that did not contaminate soil and water would reduce the incidence of hookworm which is such a prevalent and serious cause of anemia and therefore of chronic ill health in many rural tropical populations, particularly those who work in flooded rice fields. But, as pointed out elsewhere, whatever services or facilities are provided, only individual and communal understanding of their logic, purpose, and function will lead to their effective use and maintenance. But perhaps of even greater overall social significance is that only understanding of health and disease will exert the effective political pressure necessary for increased and improved health provisions, and it is a *sine qua non* if community participation is to play the critical role prescribed for it in primary health care.

Specific health education programs in industrialized countries (e.g., to stop smoking) have not been dramatically successful, but as they have become more

sensitive and culturally appropriate, their effectiveness has visibly increased. It is much less effective to threaten a teenage boy with an unpleasant death some decades away than to persuade him that girls do not like boys who smell of cigarette smoke. But how to teach populations with high rates of illiteracy and little schooling of any sort? The Brandt Commission (1980) points out that although, between 1960 and 1970, literacy increased from 65 to 75% in Latin America and from 45 to 53% in Asia, it increased only from 20 to 26% in Africa, and that 34 countries still had illiteracy rates of over 80%. And in the contexts of improving household hygiene and the bringing up of children, there is the additional impediment that in most of these populations the education of women and girls lags far behind that of men and boys.

Posters obviously vary in their effectiveness with literacy and also with comprehensibility: one often sees posters in a national (but not local) language using words that challenge even the highly literate, and they need the highest skills of the advertiser as well as of the educationalist. Cartoons can be very effective if conceived and drawn with similar skills, but the language should if possible be local. They must be culturally and socially appropriate: those appropriate for urban populations may be poorly understood by rural people, and vice versa. But the transistor radio is now to be found even in quite remote villages, and village television sets are provided in at least some parts of some countries. These media can ignore illiteracy provided that they use a generally understood language and an educationally, culturally, and socially appropriate approach. The understanding of biology and disease among village people is often greatly underestimated, and although many infection and disease concepts that need to be understood are scientifically sophisticated, they can be adequately explained if sufficient effort is made by more educated members of the same community who have had adequate guidance. Few countries fail to use every available skill to promote the government's political views through their media; the same resources, better utilized and with additional skills, might, over time, produce health benefits quite disproportionate to those obtainable by similar expenditure on more conventional health budget items.

Although health education should clearly play an important part in school education, and that kind of teaching can be deliberate, more subtle methods may be more effective through public media. At least some people do not respond to attempts to lecture them into changes in behavior, and the subtle use of stories and drama, particularly ones they can identify with in their daily lives, with reiterative messages about health and behavior intermingled may be much more effective. In the West, large populations become addicted to and emotionally involved in serial radio and television dramas, and cultural and social equivalents appropriate to developing countries might be similarly habit forming.

Whatever method is employed, its effectiveness will not be known, and it will not be possible to systematically improve it, unless it is skillfully evaluated.

This is another area of endeavor where the methodology for health has hardly been created and where trained personnel scarcely exist. The skills of market research, advertizing, and politics will need to be converted and harnessed to health.

The priorities for health education, then, are to create a number of centers with staff from a variety of linguistic and cultural backgrounds and with health skills alongside advertising, media, and epidemiologic skills to develop the technology of education and its evaluation and then to train nuclei for a wider and wider distribution of units concerned with promoting and evaluating health education at national and more local levels.

3.2. More Self-Reliant Policy Development

The range of new and desirable initiatives that are now competing for available technical and financial resources both in developing and industrialized countries include "Health for All by the Year 2000," the "Drinking Water and Sanitation Decade," and the Special Programmes for Research and Training in Tropical Diseases and for the Control of Diarrhoeal Diseases, and these against a background of many longer-standing or less adventurous but no less important activities: family planning, maternal and child health, the WHO Expanded Immunization Programme, and the like. All this must be done during a period of world recession from which slow recovery seems likely and during which the developing countries will have many priorities other than health to contend with in their efforts to accelerate development. A prerequisite for meeting these challenges is the creation of such scientific self-reliance that a country can understand and measure its own problems, can make optimal use of its own resources (whether large or small) to solve its own problems by applying all available and relevant knowledge in nationally appropriate cultural, social, political, economic, and ecological terms, can formulate and take initiatives to seek and obtain the most appropriate outside technical and financial assistance to solve the problems it has identified and given priority to, can collaborate effectively on terms of mutual respect with other countries, developing and industrialized, and can evaluate the effectiveness of its own policies and of the measures taken to implement them.

In the health sector, the implementors and skills that tropical developing countries need to interpret information, to solve problems, and to successfully integrate and communicate them to policy makers include, as well as doctors and nurses, biologists (entomologists, malacologists, and the like), nutritionists, engineers (water and sanitation), epidemiologists and demographers to estimate the resources needed and to measure the effects of interventions to improve health, health economists and operational research to evaluate and improve the imple-

mentation of policies, and the physicists, chemists, agriculturalists, and legislators who are responsible for the environment.

Many Third World countries already have scientists in several of these areas of expertise, often despite a serious "brain drain" resulting from lack of appropriate policies to retain such trained manpower. But there is often a serious lack of useful dialogue between these scientists and their governments. When faced with a need for advice, governments often prefer to turn to a foreign consultant lacking knowledge of the country, its problems, its customs, its ecology, and even its health problems. They know that in asking for outside technical assistance or financial aid their case may be stronger if they can say they have sought (if not, sometimes wisely, accepted) the advice of one or more internationally known "experts." But neither these governments nor those who seek to aid them can have the best advice unless they create and effectively use their own national sources of knowledge and experience. They need to ensure that selected scientists are given not only training but relevant experience, and the scientists themselves must make the necessary efforts to become more credible sources of advice. Only then will governments be able to use outside technical and financial assistance to their own best advantage.

3.3. A Scientific Basis for Policies and Their Implementation

If governments are to have the continuing dialogue they need with scientists in the formulation, evaluation, and refinement of policy, some structure is necessary within which this can occur. Although it should not have a permanently fixed membership, it must have sufficient continuity to develop into a thinking body, and it must incorporate both those who are responsible for implementing policy (whether or not they are scientists) as well as scientists from relevant universities or research institutions. On the other hand, it should not be so enmeshed at the political level that, should there be a change of government, nearly all the national scientific expertise would be ineligible to assist. Stocking and Smith (1981) examined these issues and recommended that more attention be given to providing relevant experience for the scientists (particularly those responsible for government programs) in projects and programs both in their own and similar countries. To achieve this, a major change in attitude by donor agencies would be needed so that maximal use was made of developing country scientists in program planning and management. This would be easier if such programs and projects were of a more easily manageable size. They considered that this closer involvement of nationals would, moreover, enhance the chances of programs and projects leaving self-sustaining gains behind them.

Incentives would have to be provided to members of the government advisory structure (probably financial and preferably pensionable) so that they would identify strongly with it, and membership should provide such status that

it would itself act as a career incentive for scientists to aspire to. The nature of the structure would have to be conceived for each country by itself and would obviously differ in different countries. Some developing countries already have National or Medical Research Councils or Academies of Science, but these are conceived for a different purpose. The Research Strengthening Group has recognized their importance and is endeavouring to strengthen them. Their membership and terms of reference might be modified to fit the role suggested here, but, because they are widely seen as concerned with research, it will probably be more effective to develop new structures much more concerned with implementation, evaluation, and management of government policies.

3.4. Training, Experience, Opportunities, and Careers

If training is to be effective in creating a national cadre of scientific expertise or in strengthening research and training institutions, those who are trained must be able to perceive, in their countries, opportunities to utilize their expertise effectively and to develop their capabilities to their fullest extent, and there must be satisfactory career prospects. If scientists are to achieve their full potential, it is not enough to give them a course—their whole career development needs to be planned, including job experience under mature guidance or apprenticeship to a more experienced scientist and refresher training and further experience as required.

Because they do not offer satisfactory job opportunities, careers, or salaries (and this is the order of priority), many countries fail to retain their trained (and especially their more gifted) scientists. Moreover, governments often fail to identify training with job requirements: a post necessary to development should be identified, an apt candidate chosen and trained and then established in the appropriate post. This process is frequently lacking in one or several respects and is a major cause of "brain drain." The Brandt Commission (1980) estimated that in the 1960s and 1970s well over 400,000 physicians and surgeons, engineers, scientists, and other skilled people moved from developing countries to more developed ones.

Certain important types of expertise are, however, rare worldwide, and there is a need to create foci within which the subject could be developed and scientists trained. Important examples are operational research and economics as applied to health problems, environmental engineering relevant to the tropics, scientists trained to develop food policy in the interfaces between nutrition, agriculture, and economics, and population scientists able not only to advise on family planning and infertility but also to interpret the fragmentary statistics available so as to make effective projections for planning of all sorts, and the expertise for effective health education. These skills are scarce at a high level even in the industrialized countries, and it will not be enough merely to provide

funds for training and research. There needs to be special funding to create the academic nuclei in which ideas, methodology, and training programs can be developed. The germinal centers for these subjects can best be created in institutions that offer a comprehensive view of health worldwide and attract a highly international student body. From such centers trained scientists could develop their own national centers throughout the developing countries, perhaps first on a regional basis.

3.5. Research

The research needed to solve the problems of health in the tropics falls into two main classes: research aimed at more fundamental issues, particularly of a technological or methodological nature, which can be pursued anywhere in the world; and research into the ecological, epidemiological, and sociological aspects of disease, into the application of new knowledge, and into the implementation and evaluation of possibly beneficial interventions. These latter areas of research have to be pursued where the disease occurs and is transmitted. Both kinds of research are supported by the WHO Special Programmes, by major foundations such as the Rockefeller Foundation and the Wellcome Trust, and by many other bodies, national and international.

In the particular case of research directed at the discovery of new drugs or vaccines, at least their development demands the strong involvement of the pharmaceutical industry, and in general their systematic discovery is more likely by the disciplined approaches of industry. However, as the cost of research and development leading to a drug or vaccine of fully established efficacy and safety may require expenditure as high as \$20 million, it is not surprising if industry does not direct its fullest possible efforts against diseases confined or largely confined to developing countries where sales seem unlikely to recover these costs. Thus, either international agencies must find ways of assisting financially in the development of promising drugs or vaccines for major diseases or aid-giving countries should provide some of their aid by helping their own industries to contribute more effectively. When it comes to deploying such drugs or vaccines in the vast poor populations who need them, there needs to be available the expertise in the developing countries to carry out definitive clinical trials and to develop effective plans and infrastructures to administer the drugs or vaccines. In the case of vaccines, an effective "cold chain" right down to the periphery is also necessary, and the WHO Expanded Immunization Programme is having considerable successes in helping developing countries to establish their country wide cold chains.

The shortage of epidemiological skills is an important barrier to progress with the planning, implementation, and evaluation of major disease control programs in nearly all developing countries. If the information is to be available

for planning such interventions and for evaluating them, the developing countries will need to develop effective disease surveillance programs that can, in addition, identify outbreaks and epidemics of disease and bring control measures to bear on them. An outstanding example of the sort of organization and resources required is provided by the Caribbean Epidemiology Centre at Port of Spain, Trinidad, which provides training, laboratory back-up, and a "fire-brigade" service for the surveillance and control of infective diseases throughout much of the Caribbean (Hamilton and Diggory, 1979).

In terms of drugs and vaccines (Fattorusso, 1979), it is also important to recognize that, because of the lack of effective quality control, many developing countries purchase drugs and vaccines of poor quality, stability, and effectiveness. This serious waste of resources will only be overcome by the development of national or regional centers capable of assaying the quality of drugs and vaccines and thus permitting governments to be assured of the quality of what they purchase.

Many developing country governments fear escalating demands for research, and because of poorly comprehensible justification they may fail to see it as a reasonable burden in face of many competing demands; and scientists seem no sooner to have solved one problem than they expose an apparently endless series of further problems to solve. In the context of development, research has three clear and important contributions to make. (1) The problems of developing countries are different from those of industrialized countries, as are the solutions for differing ecological, social, cultural, and political contexts. Confidence in the solutions will, of course, depend on the scientific rigor with which they have been investigated and thus on the quality and training of the scientists concerned. (2) Research is an essential context for effective higher education: it creates an environment in which inquiring minds in both staff and students can be stimulated and trained. (3) It provides opportunities for the best minds in any country to realize their full potential. Third World scientists may find it easier to convince their governments of the value of their research if they are more prepared to take the trouble to clearly define its purpose under one or more of these headings and to explain, in terms comprehensible to people outside their discipline, how it will advance development of their country. If possible, their research should be seen to take maximum advantage of the unique or special opportunities offered by their own country—more often in the field than the laboratory. This gives them a clear advantage over perhaps better funded competitors who lack such opportunities. They should beware of the false values that classify the solution of practical and important national problems as either less intellectually challenging or less scientifically "respectable" than the abstract pursuit of knowledge.

The attitude of Third World governments towards research varies greatly, but it is already evident that the WHO Special Programme for Research Training in Tropical Diseases has generated a very considerable interest, and their re-

sponses to taking over additional resources created by the RSG is already encouraging. The RSG was set a task different from any previously attempted on a worldwide scale and has developed its policy step by step, gaining experience as it goes along. Its objective is to assist developing countries to assume their appropriate roles in carrying out research to identify, analyze, and solve health problems caused by the six diseases. Its activities are designed to stimulate and complement national activities in research in accordance with the country's needs and policies and ability to financially sustain them within a reasonable period. The institutions and governments concerned must, therefore, themselves formulate the objectives toward which they wish to be helped.

4. CONCLUSION

The health problems of the Third World are complex and almost overpowering in their scale. It is easier to prescribe solutions for these ills than to persuade those who can solve them to take the medicine that has to be taken not only by the developing countries themselves but also by those countries who must help them. The messages of the Brandt Commission (1980) are loud and clear, and whether or not it prescribes the right solution, North and South together must find the answers "guided by mutual respect, openmindedness and honesty, with a willingness not only to offer criticism but also to listen to it." The message to researchers, to those who support them, and most of all to those who need to use their discoveries is no different from that given by Sir Ronald Ross in his Presidential Address to the Royal Society of Tropical Medicine and Hygiene more than 70 years ago (Ross, 1909):

Do not think that when you have made your discovery, great or small, you have finished the matter. Medical research is not a mere academic amusement consisting in the publication of elegant articles adorned with coloured plates. The discovery is only half-way up the mountain, and beyond it extends the arduous summit of the practical application.

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REFERENCES

- Abdel-Wahab, K. S. E., El Baz, L. M., El Tayeb, E. M., Omar, H., Ossman, M. A. M., and Yasin, W., 1978, Rift Valley fever virus infections in Egypt: Pathological and virological findings in man, *Trans. R. Soc. Trop. Med. Hyg.* 72:392-396.
- Ashcroft, M. T., and Desai, P., 1978, Blood-pressure and mortality in a rural Jamaican community, *Lancet* 1:1167-1170.
- Berge, T. O. (ed.), 1975, International Catalogue of Arboviruses Including Certain Other Viruses of

- Vertebrates, 2nd ed., U.S. Department of Health Education and Welfare Publication No. (CDC) 75-8301.
- Boshell, M. J., 1969, Kyasanur Forest disease: Ecological considerations, *Am. J. Trop. Med. Hyg.* 18:67-80.
- Bouvier, L. F., 1980, A "numbers" approach to the 1980 data sheet, *Intercom* 8(4):8-10.
- Brandt Commission, 1980, *North-South: A Program for Survival*, MIT Press, Boston.
- Browne, S. G., 1980, Le contrôle de la lèpre. Chimères et possibilités, *Bull. Mem. Acad. R. Med. Belg.* 135:208-218.
- Catterall, R. D., 1981, Biological effects of sexual freedom, *Lancet* 1:315-319.
- Chambers, R., Longhurst, R., Bradley, D., and Feacham, R., 1979, Seasonal dimensions to rural poverty: Analysis and practical implications, *J. Trop. Med. Hyg.* 82:156-172.
- Cockburn, W. C., 1971, World health importance of certain infectious diseases, in: *Proceedings, International Conference on the Application of Vaccines against Viral, Rickettsial and Bacterial Diseases of Man*, Pan American Health Organization Scientific Publication No. 226, PAHO, Washington, pp. 3-11.
- Costa, M. F. F. de L., Katz, N., and Dias, J. C. P., 1980, Reinfecção de pacientes em áreas de esquistossomose mansonii após tratamento específico. IV. Observações em Belo Horizonte, *Rev. Inst. Med. Trop. Sao Paulo* 22:97-107.
- Davidson, G., Herath, P. R. J. and Hemingway, J., 1980, Recent trends in insecticide resistance shown by anopheline mosquitoes, in: *10th International Congress on Tropical Medicine and Malaria. Manila, Philippines, November 9-15, 1980. Abstracts*. Quezon City: International Congress on Tropical Medicine and Malaria, 1980, pp. 68-69.
- Davidson, G., Herath, P. R. J., and Hemingway, J., 1980, Recent trends in insecticide resistance shown by anopheline mosquitoes, in: *Abstracts 10th International Congress on Tropical Medicine Mal. Manila*, pp. 101-102.
- Echeverria, P., Verhaert, L., Ulyangco, C. V., Komalarini, S., Ho, M. T., Orskov, F., and Orskov, I., 1978, Antimicrobial resistance and enterotoxin production among isolates of *Escherichia coli* in the Far East, *Lancet* 2:589-592.
- Fattorusso, V., 1979, Problems of distribution, availability and utilization of agents in developing countries. D. Developing country perspectives—an overview, in: *Pharmaceuticals for Developing Countries*, National Academy of Sciences, Washington, pp. 250-259.
- Feachem, R. G., 1978, Domestic water supplies, health and poverty: A brief review, *Water Supply Manage.* 2:351-362.
- Fendall, N. R. E., 1965, Medical planning and the training of personnel in Kenya, *J. Trop. Med. Hyg.* 68:12-20.
- Food and Agriculture Organization, 1979, *Nutrition in Agriculture, Committee on Agriculture Vth Session*, FAO, Rome.
- Georgioui, G. P., 1972, Studies on resistance to carbamate and organophosphorus insecticides in *Anopheles albimanus*, *Am. J. Trop. Med. Hyg.* 21:797-806.
- Golliday, F. L., and Koch-Waser, C. K., 1977, The new politics for rural health: Institutional, social and financial challenges to large scale implementation, in: *Technologies for Rural Health*, Proceedings B, Vol. 199, The Royal Society, London, pp. 169-178.
- Guillet, P., Escaffre, H., Ouedraogo, M., and Quillévéré, D., 1980, *Note Préliminaire sur une Résistance au Temephos dans le Complexe Simulium damnosum (S. sanctipauli et S. soubrense) en Côte d'Ivoire (Zone du Programme de Lutte Contre l'Onchocercose dans la Région du Bassin de la Volta)*, WHO3VBC380.784, World Health Organization, Geneva.
- Halstead, S. B., 1966, Mosquito-borne haemorrhagic fevers in south and southeast Asia, *Bull. WHO* 35:3-15.
- Halstead, S. B., 1970, Observations related to pathogenesis of dengue haemorrhagic fever. VI. Hypotheses and discussion, *Yale J. Biol. Med.* 42:350-362.

- Hamilton, P., and Diggory, P., 1979, The Caribbean Epidemiology Centre (CAREC), *Bull. Pan Am. Health Org.* 13:187–194.
- Haub, C., 1980, World population: The next 20 years, *Intercom* 7(10):8–9.
- Herath, P. R. J., and Davidson, G., 1981, Studies on the nature of malathion resistance in a population of *Anopheles culicifacies* Giles, *Bull. WHO*, 59(3):383–386.
- Joshua, G. E., Ratnaik, N., and Benjamin, V., 1971, Lead poisoning in a family of 18 members in Vellore town, *Indian J. Med. Res.* 59:1496–1507.
- Lancet, 1981, International Drinking Water Supply and Sanitation Decade, *Lancet* 1:321.
- Longhurst, R., and Payne, P. R., 1981, Seasonal aspects of nutrition, in: *Seasonal Dimensions of Rural Poverty: Analysis and Practical Implications* (R. Chambers, R. Longhurst, D. Bradley, and R. Feachem, eds.), Frances Pinter Ltd., pp. 45–52.
- Lovemore, D. F., and Bax, P. N., 1972, An isolated outbreak of cattle trypanosomiasis near the Victoria Falls, Rhodesia, *Rhodes. Sci. News* 6:14–17.
- Mara, D., and Feachem, R., 1980, Technical and public health aspects of low cost sanitation programme planning, *J. Trop. Med. Hyg.* 83:229–240.
- Marques, A. C., 1979, Migrações internas e as grandes endemias, *Rev. Bras. Malarial Doenças Trop.* 31:137–158.
- Menon, P. K. B., and Rajagopalan, P. K., 1980, Relative importance of different types of breeding habitats in contributing to the population of *Culex pipiens fatigans* in Pondicherry, *Indian J. Med. Res.* 71:725–733.
- Monath, T. P., 1975, Lassa fever: Review of epidemiology and epizootology, *Bull. WHO* 52:577–592.
- Puffer, R. R., and Serrano, C. V., 1973, *Patterns of Mortality in Childhood*, Pan American Health Organization Scientific Publication No. 262, PAHO, Washington.
- Ramsdale, C. D., Herath, P. R. J., and Davidson, G., 1980, Recent developments of insecticide resistance in some Turkish anophelines, *J. Trop. Med. Hyg.* 83:11–19.
- Retel-Laurentin, A., 1967, Influence de certaines maladies sur la fécondité: Un exemple africain, *Population* 22:841.
- Ripert, C., Carteret, P., and Gayte, M. J., 1969, Étude épidémiologique des bilharzioses intestinales et urinaire dans la région du lac de retenue de la Lufira (Katanga), *Bull. Soc. Pathol. Exot.* 62:571–581.
- Rosen, L., 1977, The emperor's clothes revisited, or reflections on the pathogenesis of dengue hemorrhagic fever, *Am. J. Trop. Med. Hyg.* 26:337–343.
- Ross, R., 1909, Presidential address: The future of tropical medicine, *Trans. R. Soc. Trop. Med. Hyg.* 2:272–285.
- Schenone, H., 1971, Estado de los estudios epidemiológicos sobre la enfermedad de Chagas en Chile, *Bol. Of. Sanit. Panam.* 70:250–255.
- Singh, D., 1967, The *Culex pipiens fatigans* problem in South-East Asia: With special reference to urbanization, *Bull. WHO* 37:239–243.
- Smith, C. E. G., 1964, Factors in the transmission of virus infections from animals to man, in: *Scientific Basis of Medicine*, Annual Reviews, Athlone Press, London, pp. 125–150.
- Smith, C. E. G., 1975, Changing patterns of disease in the tropics, in: *Man-Made Lakes and Human Health* (N. F. Stanley and M. P. Alpers, eds.), Academic Press, London, pp. 345–362.
- Smith, C. E. G., 1978, “New” viral zoonoses: Past, present and future, in: *Microbial Ecology* (M. W. Loutit and J. A. R. Miles, eds.), Springer-Verlag, Berlin, pp. 170–174.
- Sofoluwe, G. S., Adegbola, A., and Akinyani, P. A., 1971, Urinary delta-aminolevulinic acid: Determinations among workers charging lead accumulator batteries in Lagos, Nigeria, *Arch. Environ. Health* 23:18–22.
- Stocking, B., and Smith, C. E. G., 1981, National policies and national experts: Basic requirements in developing countries, *Lancet* 1:144–145.

- Tayo, M. A., Pugh, R. N. H., and Bradley, A. K., 1980, Malumfashi Endemic Diseases Research Project, XI. Water contact activities in the schistosomiasis study area, *Ann. Trop. Med. Parasitol.* 74:347–354.
- Thiruvengadam, K. V., Kalyanasundaram, V., and Rajagopal, J., 1965, Clinical and pathological studies on chikungunya fever in Madras City, *Indian J. Med. Res.* 53:729–744.
- Thylefors, B., and Tønjum, A. M., 1980, A three-year follow-up of ocular onchocerciasis in an area of vector control, *Bull. WHO* 58:107–112.
- United Nations, 1980, *World Population Trends and Policies, 1979 Monitoring Report*, Volume 1, *Population Trends*, United Nations, New York.
- UNDP/World Bank/WHO, 1979, *UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, Third Annual Report, 1 July 1978–30 June 1979*, WHO, Geneva.
- Valverde, V., Vargas, W., Payne, P., and Thomson, A., 1981, Data requirements and use in nutrition planning in Costa Rica, *Food Policy* 6:19.
- Vaughan, J. P., 1980, Barefoot or professional? Community health workers in the Third World, *J. Trop. Med. Hyg.* 83:3–10.
- Walsh, J. F., 1972, Blackflies entering vehicles in northern Nigeria, *Trans. R. Soc. Trop. Med. Hyg.* 66:803–805.
- Walsh, J. F., Davies, J. B., and La Berre, R., 1979, Entomological aspects of the first five years of the Onchocerciasis Control Programme in the Volta River basin, *Tropenmed. Parasitol.* 30:328–344.
- Waterlow, J. C., and Payne, P. R., 1975, The protein gap, *Nature* 258:113–117.
- World Bank, 1975, *Health, Sector Policy Paper (Health Budgets)*, World Bank, Washington.
- World Bank, 1979, *World Development Report, 1979*, World Bank, Washington.
- World Bank, 1980, *Water Supply and Waste Disposal, Poverty and Basic Needs Series 1980*, World Bank, Washington.
- World Health Organization, 1972, Occupational health programmes, *WHO Chron.* 26:537–546.
- World Health Organization, 1975, Control of nutritional anaemia with special reference to iron deficiency, *WHO Tech. Rep. Ser.* No. 580.
- World Health Organization, 1978a, *The Work of WHO 1976–1977, Biennial Report of the Director General, WHO Official Record No. 243*, WHO, Geneva.
- World Health Organization, 1978b, *Primary Health Care*, WHO, Geneva.
- World Health Organization, 1979a, Advisory group on the prevention of blindness: Data on blindness throughout the world, *WHO Chron.* 33:275–283.
- World Health Organization, 1979b, Diarrhoeal Diseases Control Programme, *Weekly Epidemiol. Rec.* 54:393–395.
- World Health Organization, 1980a, Resistance of vectors of disease to pesticides, fifth report of the WHO Expert Committee on Vector Biology and Control, *WHO Tech. Rep. Ser.* No. 655.
- World Health Organization, 1980b, Viral respiratory diseases, report of a WHO scientific group, *WHO Tech. Rep. Ser.* No. 642.

African Trypanosomiasis

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1. THE DISEASE IMPACT

African trypanosomes are spread by tsetse flies (*Glossina*) over about 10 million square kilometers of intertropical Africa where they cause disease in man and his domestic animals.

The number of new sleeping sickness patients recorded annually amounts to not more than 10,000; however, it is estimated that 35 million people are at risk and that the actual number of cases may lie anywhere between this figure and the 10,000 cases officially recorded (De Raadt, 1975).

The economic impact of the animal disease is mainly on cattle, although other domestic animals such as goats, sheep (Griffin, 1978), and camels are also involved. It is estimated that 7 million square kilometers in Africa with high-quality pastures and abundant water could be used for cattle raising were it not for animal trypanosomiasis (Finelle, 1974, 1980). This area would be sufficient to support another 120 million cattle which would provide 1.5 million metric tons of meat per year (Finelle, 1974). To this loss of resources should be added the high cost of chemotherapy and chemoprevention in certain areas where some cattle raising is possible because of lower fly challenge.

Human trypanosomiasis is one of the major diseases selected by the World Health Organization, the United Nations Development Programme, and the World Bank for their joint Special Programme for Research and Training in Tropical Diseases. Similarly, considering the magnitude of the animal problem, the 1974 World Food Conference of the United Nations gave the Food and Agriculture Organization the mandate "to launch, as a matter of urgency, a long-

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term program for the control of African animal trypanosomiasis as a project of high priority'' (reported by Finelle, 1980).

The purpose of this review is to focus on new developments in African trypanosomiasis of man and domestic animals. We will briefly describe the various clinical syndromes of the disease caused by *Trypanosoma* (*Trypanozoon*) *brucei gambiense* or *rhodesiense* in man and *T. (Trypanozoon) brucei brucei*, *T. (Nannomonas) congolense*, or *T. (Duttonella) vivax* in cattle.

In man, the disease starts with elevated temperature and local chancre at the site of the tsetse fly bite. Later, there are irregular fever, adenopathies, hepatosplenomegaly, digestive troubles, sometimes with dysenterylike diarrhea, kidney malfunction with oliguria and albuminuria, cardiomegaly, and finally nervous system disturbance resulting from diffuse meningoneuraxitis. Eventually the nervous system problems become prominent with hyperesthesia, muscular fibrillation, various paralyses, cerebellar and extrapyramidal signs, delirium, epileptic crisis, and spectacular sleep disorder with first sleepiness and later somnolence accompanied by invincible intellectual apathy. Patients, in the absence of treatment, die in marasmus from nervous and cardiovascular collapse. The disease is fatal in a few months to 2 years.

In cattle, the disease course may take as long as several years, with some animals recovering spontaneously and others dying at widely different times after onset. Differences in susceptibility are both individual and related to breed of cattle. The pathology of the cattle disease has been described since the last century (Kanthack *et al.*, 1898; Hornby, 1921; Hu, 1931) with precious few recent changes (Goodwin, 1970; Murray, 1974; Murray, *et al.*, 1980). It appears that ''variation in the pathological changes depends largely on the duration of the infection, rather than the kind of parasite or host'' (Hu, 1931).

Moreover, it seems that anemia leading to heart failure is the single most important pathogenic factor (Anossa and Isoun, 1976; Murray, 1979; Murray *et al.*, 1980). The mechanism of anemia was studied in depth by Dargie *et al.* (1979) and appears to be caused by excessive hemolysis, erythrophagocytosis, and hemosiderosis with some degree of marrow disfunction. Amole *et al.*, (1981) reported that there are different mechanisms of anemia during the early acute and late chronic phases of a *T. brucei brucei* infection in mice. In the acute phase, there is primarily sensitization of erythrocytes by IgM immune complexes which is partially compensated for by erythropoiesis; in the chronic phase, however, intense anemia is caused by sequestration of erythrocytes in spleen and liver accompanied by hemodilution as well as the continuing erythrocyte sensitization by IgM immune complexes. There is also a gross involvement of the lymphoid system with spectacular enlargement of spleen and lymph nodes, disruption of these organs' normal architecture, proliferation of normoblasts and lymphoblasts, and appearance of large numbers of plasmocytes and macrophages at the height of the disease. Later on, the lymphoid organs become depleted of lymphoid cells.

It should be emphasized that both human and cattle trypanosomiasis are long-lasting chronic diseases, and the outcome in cattle is not always fatal. This makes them quite different from the disease induced by the same parasites in small rodents which are often used as experimental models; in these, the disease course is acute, and the outcome always fatal, often in a matter of days.

It is also worth mentioning that trypanosomes pathogenic for man or cattle may be found in wild animals where they apparently cause no harm (Cunningham, 1968; Geigy *et al.*, 1973a,b; reviewed in De Raadt, 1975) and that a human pathogen, *T. brucei rhodesiense*, may be found in cattle (Mwambu *et al.*, 1975). Moreover, many other species of trypanosomes exist which infect a wide variety of hosts, often with mild or no consequences (Hoare, 1972). Clearly, not all parasite-host pairs have achieved the same level of "partner integration" (Margulis, 1976). Of great pathogenic importance, however, is *T. cruzi*, the agent of human South American trypanosomiasis or Chagas' disease; this is an intracellular parasite, unlike the African trypanosomes, which has diverged from the African species long ago as judged by their respective $(A + T)/(G + C)$ ratios (Bouisset, 1968).

From early on, following Pasteur's successes, vaccination attempts were made. Kanthack *et al.* reported as early as 1898 their failure with 13 different attempts to produce immunity. Laveran (1911) reported some success in a few goats and sheep infected with weak pathogens, although no pathogenicity controls were performed, but, unfortunately, even today, one can only agree with Van Saceghem's (1938) conclusion, "C'est que les bovidés même guéris après traitement ne possèdent aucune immunité et peuvent donc toujours se réinfecter," and Hornby's (1941) "Twenty years ago I concluded that in some districts a number of native cattle recover spontaneously from infection but that no great immunity results therefore (Hornby, 1921), and I still hold the same opinion."

It is the approach of this review to analyze why immunity does not appear to "work." We first examine two alternatives to immunization, vector eradication and drug treatment, before analyzing the problems the immune system has to overcome during trypanosome infections. We then consider the role of the immune system and the genetic basis for susceptibility differences to trypanosomiasis. We conclude by giving some tentative prospects.

2. PROBLEMS OF ERADICATION

2.1. Vector Control

Here is not the place to describe at length the various elaborate procedures that have been used to try to eradicate *Glossinas*. But since these obviously would present, if successful, an alternative to drug protection or vaccination, they need to be surveyed. Detailed treatment of this subject can be found else-

where (Mulligan, 1970), and many developments are reported in a recent symposium (International Atomic Energy Agency, 1980). There is also an excellent review by Molyneux (1976) on vector relationship in the *Trypanosomatidae*.

2.1.1. Synthetic Chemical Pesticides

Pesticides have been used on a large scale to eradicate tsetse flies. Although the method has been successful in temporarily lowering the tsetse fly population in defined areas, it has been hampered by many factors: persistence of residual foci of flies in areas not sprayed or not accessible, repopulation of cleared areas by residual flies not killed by insecticide, possibility of the establishment of resistance to pesticides, although this has been estimated to be still mild in the case of tsetse flies, unwanted side effects of pesticides on man, animals, and the environment, and, last but not least, the gigantic effort of coordination for maintenance and surveillance of the eradication program at the level of a continent. It has been estimated that clearance by pesticides may cost from U.S. \$20–50 to \$4,500 per square kilometer depending on the terrain and the fly species tackled (Finelle, 1974). Clearly, to eradicate flies by pesticide may be feasible in specific locations, but treating successfully the 10 million square kilometers covered with tsetse flies at a cost that might be as high as U.S. \$20,000 million appears to be utopic.

2.1.2. Release of Sterile Males

This technique consists in releasing sterile males in an area where they would compete with wild males for mating females.

This approach has been used successfully for the eradication of the screw-worm fly from the entire southeastern United States (reported by Mulligan, 1970). Two major experimental stations, one in Upper Volta and the other in Tanzania, have investigated the feasibility of this technique for tsetse flies (Itard and Clair, 1975; Cuisance *et al.*, 1978, 1980; Politzar *et al.*, 1980; Dame *et al.*, 1980).

It appears from the Bobo-Dioulasso data that weekly release starting during the dry season at 1-km intervals along gallery forests so as to achieve a ratio of sterile to wild males of 10:1 might be successful in eradicating *Glossina palpalis gambiensis* if the starting fly population is not too high or has been lowered by prior insecticide treatment (Cuisance *et al.*, 1980). Considering the cost of each sterile male, about U.S. 20 cents, and the average release required which is estimated at 1000 males per week per kilometer of gallery forest, the operation would cost about U.S. \$10,000 per year per kilometer or about \$140,000 for the 14 km reported eradicated of *G. palpalis gambiensis* so far. This area still harbors *G. tachinoides*.

In the Tanga project, after two pesticide aerial sprayings on a 200-km² test plot which reduced the *Glossina* populations (*G. morsitans morsitans* and *G. pallipides*) by more than 95% on each application, *G. morsitans morsitans* irradiated pupae were distributed at 120 release sites twice weekly at a number averaging 135 sterile males per month per site. The *G. pallipides* returned to the levels they had before insecticide spraying within 3 months, while the *G. morsitans morsitans* population remained, for a period of about 1 year, at about one-tenth of the level it was before spraying. Each sterile male cost 22 cents, and so the low levels of *G. morsitans morsitans* (with usual levels of *G. pallipides*) on the 200 square kilometer plot were obtained at a cost in flies alone of U.S. \$71,280 per year. One must remember that the area at risk covers 10 million square kilometers of Africa.

It is hoped by workers in this field that the possibility, presently under investigation, of mass maintenance of tsetse flies in Africa by an *in vitro* feeding technique, e.g., by the use of γ -irradiated blood (Bauer *et al.*, 1980) would alleviate the restraint on the number of sterile males produced at present and reduce their production cost.

2.1.3. Trapping of Tsetse Flies

Mass trapping of tsetse flies by hand has been suggested, and it could considerably improve the fly load in certain specific geographic and demographic conditions. Traps have been used for epidemiological studies and control operations. The most practical model seems to be that introduced by Challier and Laveissière (1973) and whose modified version has been recently described in detail by Ryan and Molyneux (1980). Incorporation of host odors to increase efficiency has been reported (Ryan and Molyneux, 1980), and the use of pheromones has been suggested.

2.1.4. Clearance of Tsetse Fly Habitat and Wild Animal Reservoir

Clearance of gallery forest over sections of a few hundred meters is commonly used to establish a barrier to reinfestation, but it appears that such barriers are not very efficient and that tsetse flies cover longer distances than was previously thought, up to at least 28 km (J. Février, D. Cuisance, and H. Politzar, personal communication), and can be carried long distances by wind (Molyneux *et al.*, 1979).

The systematic destruction of vegetation and forests as well as the slaughtering of all wild animals in Africa have been suggested and even partially tried (reported in Mulligan, 1970, pp. 543–562). This approach has been universally condemned by even the least ecologically minded people and is only mentioned here for horror value.

2.1.5. General Remarks

Besides the methods described, it has also been suggested to use tsetse fly predators or pathogens (Laird, 1975), but we are unaware of any field trial in this direction.

None of the vector eradication methods taken singly or in combination appears to be able to bring a general solution to the overall problem of trypanosomiasis in Africa, although they may be efficient in defined local conditions. One also cannot help but wonder about the fabulous cost. Moreover, one should also mention that trypanosomes can be mechanically transmitted by insects other than tsetse flies, especially *Stomoxys*, *Tabanidae*, and *Culicinae* (reviewed in Wells, 1972), although no known endemy has been reported in Africa in the total absence of tsetse flies. Additionally, in South America, *Trypanosoma vivax* might well have adapted itself to development in another arthropod and be cyclically transmitted in the total absence of *Glossina* (Wells, 1972).

2.2. Drug Treatment

A second alternative to immunization is the early diagnosis of human and animal cases of trypanosomiasis followed by treatment. Better yet would be the systematic chemoprevention as has been made possible for malaria by quinine derivatives.

2.2.1. Sleeping Sickness in Man

Let us say from the onset that the medical coverage of the 35 million people at risk is inadequate and that most will not have an opportunity for early diagnosis and prompt treatment. Moreover, most of the symptoms are not pathognomonic in the early phases of the disease, and treatment is not to be started lightly because of the toxic side effects of the drugs used. The best proof of infection is the detection of the parasite in blood smears, lymph, lymph node biopsy, or CSF, but this search is often cumbersome and of low sensitivity, especially in the chronic phase of the disease and in overburdened medical centers with few personnel. Simple serological tests have been developed using whole, sonicated, or lyophilised trypanosomes as antigen (reviewed by De Raadt, 1975). More recently, an adaptation of the Enzyme-Linked Immunosorbent Assay (ELISA) was introduced (Luckins, 1977) as well as a card agglutination assay (Magnus *et al.*, 1978), and both seem very promising since they allow the rapid examination of large numbers of samples. Elevated IgM levels have also been used as a sign of trypanosomiasis. It is worth emphasizing that in diagnosis and treatment campaigns, often using mobile units in the field, speed is of paramount importance to insure that positive cases will still be available for treatment. Sending back samples to a central laboratory for diagnosis is out of the question except for epidemiologic studies.

Drugs most used in the early stages of infection in humans, that is, before central nervous system involvement, include suramin, pentamidine, and diminazine aceturate (Berenil®).

Suramin seems to be effective against both *T. rhodesiense* and *T. gambiense*, and the efficiency is regarded as near 100%. Side effects to suramin include a febrile reaction, sometimes with vomiting, shock, and even death after the first injections; furthermore, there are toxic reactions at the level of the skin, the eyes, the mouth, and the kidneys.

Pentamidine seems to be most effective against *T. gambiense*. The reported side effects are hypotension, hypoglycemia which may be followed by diabetes, faintness and occasional collapse, nausea, sweating, gas gangrene, and abortion.

Berenil® (diminazene aceturate, Hoechst A.G.), although introduced exclusively for use in veterinary medicine in 1955, has been tried in human trypanosomiasis (reviewed by Temu and Njogu, 1975). From cases in the literature and results of 57 early cases treated with Berenil®, these authors concluded that Berenil® is effective in treatment of early stages of human gambiense and rhodesiense trypanosomiasis, that it is easy to administer (3 doses at 5 mg/kg body weight intramuscularly at 2-day intervals) and is nontoxic, although it might cause transient albuminuria. They thus recommend Berenil® as the drug of choice for the treatment of early human cases. Although the subject is delicate, it is worth mentioning that Berenil® has not been put through the lengthy procedures and stringent requirements to obtain authorization for human use. It is thus not marketed for human medicine and cannot be prescribed by a medical doctor but only by a veterinarian for animal use.

Suramin, pentamidine, and Berenil® have no curative effect on late stages of infection with involvement of the central nervous system. For these late cases, the drug of choice seems to be melarsoprol (Mel B®, Arsobal®). The patient must be hospitalized and treated progressively with extreme caution (for details on regimen and alternative drugs, see Mulligan, 1970, Chapter 36). The drug is very irritant and may cause local thrombophlebitis as well as fever and chest and abdominal pain, but the most feared side effects on the central nervous system are “reactive encephalopathy” (10–20% of the cases) from which patients often recover and hemorrhagic encephalopathy (0.5 to 1% of the cases) which is generally fatal (D. H. H. Robertson, reported by Mulligan, 1970, p. 696).

Suramine, pentamidine, and Berenil® have some prophylactic properties for a few months, a few weeks, and at most 2 weeks, respectively. The first two could be used by persons at high risk, e.g., sleeping sickness workers in the midst of an epidemic, but are too toxic to be used for general prophylaxis.

2.2.2. Trypanosomiasis in Cattle

The most widely used curative drugs for cattle trypanosomiasis today are isometamidium chloride (Samorin®, May and Baker, Ltd., and Trypamidium®,

Specia) and Berenil®. Isometamidium gives local reactions when injected intramuscularly, and care should be taken in the choice of the site of injection so as not to diminish the meat value of the animal (Finelle, 1974).

Great hopes had been raised by introducing into cattle on a large scale in intertropical Africa drugs having long-lasting protection effects: isometamidium itself, active (3–6 months) against *T. vivax*, *T. congolense*, and *T. brucei brucei*, pyridinium bromide (Protidium®, Boots Pure Drug Co.), active (3–6 months) against *T. vivax* and *T. congolense*, and quinapyramine salts (Antrycide Prosal®t, Imperial Chemical Pharmaceutical Ltd.), active (2–3 months) against *T. brucei* and *T. evansi* (Finelle, 1973a,b, 1974). Those hopes, however, have been largely disappointed, for these three drugs very frequently select chemoresistant trypanosomes, mainly because of inadequate drug regimens (Finelle, 1973a,b). Preventive treatments should be used on a strictly controlled regimen which is often impossible in the actual conditions of traditional African cattle rearing.

Berenil®, which is rapidly excreted (Bauer, 1956), has a preventive activity that does not seem to extend for more than 3 weeks in cattle (Van Hove and Cunningham, 1964), although it might be longer in mice (at least 42 days, Lumdsen *et al.*, 1965) and in rats (28 days, Zahalsky and Weinberg, 1976). Resistance to Berenil® has been reported in Tchad, Uganda, Colombia, Kenya, and Nigeria (reviewed in Finelle, 1975) as well as Ivory Coast (E. Rosenbaum, personal communication). It appears to be more marked for *T. vivax* (MacLennan and Na'Isa, 1970).

Despite the restrictions mentioned, drug coverage might be successful in bringing economic return from raising cattle in tsetse fly-infested areas (e.g., Wilson *et al.*, 1975a,b,c.). In one experiment (Wilson *et al.*, 1975c), three groups of Boran steers were observed in a tsetse fly-infected area for 29 months. In the first group, animals were treated with Berenil® on an individual basis. In the second and third groups, the whole herd was treated either with Berenil® or with Samorin® when 5% of the animals became positive. All animals gained weight, a few died but not from trypanosomiasis, and the best economic return was obtained in the Samorin-treated group. Control animals introduced periodically without treatment in the same area all died rapidly.

2.2.3. Conclusions

In conclusion to this section, curative drugs available for human trypanosomiasis are toxic, lead to chemoresistant trypanosomes, and cannot be used for general prophylaxis. In cattle the drugs are less toxic, especially Berenil®, which has also been used for man; isometamidium chloride is used for chemoprophylaxis with some success; both drugs lead to the appearance of chemoresistant trypanosomes.

It is urgent that research be directed towards the production of new drugs against both human and animal pathogenic trypanosomes (e.g., Clarkson and

Brohn, 1976; Fairlamb *et al.*, 1977; Evans and Holland, 1978; Van der Meer *et al.*, 1979; Amole and Clarkson, 1981).

3. IMMUNOLOGIC DEFENSE

3.1. The Immune Response

As already mentioned above, attempts at vaccination against African trypanosomiasis have been made since the last century. Some claims of success were made but were rather unconvincing, since a series of strict conditions were required: right age of the animals, provenance from immunized mothers, birth at the right season, exposure to the right flies, etc. (e.g., Schilling, 1935). By and large, reports of failure were the rule (Kanthack *et al.*, 1898; Hornby, 1941). Van Saceghem (1938), however, reported that some young animals recovering from a trypanosome infection may become resistant to superinfection if they are maintained in an area where only mechanical transmission is possible.

We first review attempts to immunize by infection followed by cure and continue with various aspects of the antibody and cellular responses to trypanosomes.

3.1.1. Immunity Following Infection and Cure

It was found in both mice and cattle that immunity could be conferred by infection and cure using *T. congolense* (Browning and Calver, 1943; Fulton and Lourie, 1946; Wiesenhutter, 1970), *T. brucei* (Browning and Gulbrandsen, 1936), *T. rhodesiense*, *T. equinum*, and *T. equiperdum* (Fulton and Lourie, 1946). However, the degree of immunity conferred varied with the strain of parasite used and was strictly confined to reinoculation with the homologous trypanosome. Thus, there was no immunity if the strain used was not "stable" (Fulton and Lourie, 1946); moreover, the protection induced was of short duration, less than 35 days (Wiesenhutter, 1970).

Soltys (1955), however, reported successful long-lasting protection of Zebu cattle against *T. congolense*. Since this is the only convincing report on protection conferred by infection and drug cure, it is worth describing the work in some detail. The experiment used 23 Zebus which were exposed in an enzootic area near Mombasa, Kenya, where trypanosomiasis was predominantly caused by *T. congolense* and where the principal vector was *G. austeni*. The animals were treated prophylactically with Antrycide Prosalt® for a period of 2½ years during which none detectably contracted trypanosomiasis. For some animals the same regimen was continued, whereas for others the administration of the drug was stopped; none of the animals in either group contracted trypanosomiasis, although five fresh animals brought into the area were all infected and died. Moreover, some of the experimental animals whose treatment had been discon-

tinued were removed for 10 months from the enzootic areas to a place devoid of tsetse flies and then brought back. These animals did not become infected either for the further period of 18 months they remained under observation. At the end of the experiment, animals of all groups were injected with a strain of *T. congolense* isolated near Nairobi; no trypanosomes could be detected in any of the animals injected. Unfortunately, no infectivity control on fresh animals was included in this last part of the experiment.

Thus, practical protection can be achieved by continual exposure to tsetse flies with drug protection for a period of 2½ years, provided that the tsetse fly challenge is light and the trypanosome population not too complex. Strangely, the author could not find protective antibodies in any of the sera of the experimental cattle as detected by serum transfer to mice subsequently challenged with the homologous *T. congolense*.

The same approach was used by Wilson (1971), infecting cattle with a *T. congolense* strain or blood on which tsetse flies had fed, treatment with Berenil®, and subsequent challenge with the homologous strain of *T. congolense* or bites from tsetse flies caught in the same geographical area, respectively. The results were very confusing, with some experimental animals resisting reinfection, some dying, and some controls dying or resisting as well. In another experiment already reported above (Wilson *et al.*, 1975c, 1976), a group of Boran steers raised in a tsetse fly-infected area were treated individually with Berenil® or on a herd basis with Berenil® or with Samorin®. The range of intervals between treatments required early or late in the experiment varied marginally or not at all. Thus, no obvious resistance was developed over a 29-month exposure to tsetse flies.

In yet another experiment on a breeding herd of cattle and their calves maintained in an area of high trypanosome challenge under Berenil® protection, Wilson and colleagues (1975b) found that the interval of time needed between treatments remained approximately 33 days over a period of 2 years. Again, no resistance developed during 2 years of continuous exposure to the disease in either calves or the breeding herd. All this goes against two contradictory opinions, both reported as “well known,” that resistance to trypanosomiasis is more pronounced in young animals or that it is acquired progressively through exposure to infection. In this regard, one should mention the work of Welde *et al.* (1979) who infected Hereford cattle with the dose of 10^4 *T. congolense* organisms per 277 kg of body weight and found that 10 out of 11 animals aged 4 to 12 months self-cured, although 20 out of 20 animals aged over 2 years either died or needed drug therapy to survive.

3.1.2. Antibody-Mediated Immunity

Mesnil and Brimont (1909) had found that sera of trypanosome-infected animals could confer protection against the disease. However, these authors, as

well as Levaditi and McIntosh (1910), also found that many "races" of trypanosomes were resistant to those sera. Dodin *et al.* (1962) also demonstrated homologous protection by transfer of plasma fractions of mice or rats heavily infected with *T. gambiense*, *T. congolense*, or *T. equiperdum*. The protection was assigned to a "paraprotein" present in the globulin fraction of plasma. Seed and Gam (1966) found that the homologous protection conferred by serum passage in mice and rabbits depended on high titers of protective antibody and a challenge inoculum that was not too large. Campbell and Phillips (1976) found that injection of X-irradiated *T. rhodesiense* in C57B1/6 mice conferred protection to homologous challenge. This protection could be transferred with serum for 25 days after infection and with B cells for 10 days after infection but not with T cells.

The antibody produced in rats infected with *T. brucei* and then cured with Berenil® was essentially IgM, although on subsequent challenge it was mainly IgG as determined by 2-mercaptoethanol susceptibility (Zahalsky and Weinberg, 1976). In *T. gambiense* infection in mice, Takayanagi and Enriques (1973) showed that protection could be effected by both IgM and IgG. However, the amount of Ig that was specific for the relevant determinants of trypanosomes in each fraction was not reported. Thus, homologous protection can be achieved by antibody. This was confirmed by the work of Campbell *et al.* (1977) who showed that C57B1/6 mice that were made deficient in B cells by injection of anti- μ serum from birth had a decreased survival time and could not control the first wave of parasitemia.

Antibody appears most effective *in vivo* through agglutination of the trypanosomes by cytophilic molecules and subsequent removal by macrophages (Tizard and Soltys, 1970; Takayanagi *et al.*, 1974; Macaskil *et al.*, 1980). Takayanagi *et al.* (1974) report that engulfment of *T. gambiense* by rat macrophages is not complement dependent, and Macaskil *et al.* (1980) claim that clearance of [⁷⁵Se]-labeled *T. brucei* by mouse liver macrophages is caused by antibody-C3 complexes. *In vitro*, in the absence of macrophages, incorporation of [¹⁴C]- or [³H]leucine into a TCA-precipitable fraction of *T. rhodesiense* can be inhibited by immune serum, and this inhibition appears to be dependent on the availability of complement (Diggs *et al.*, 1976).

To delineate the role of antibody, it would be interesting to see if immune Ig protection can be transferred to any host or only to more resistant ones as is the case for protection against the intestinal nematode *Trichuris muris* (reviewed in Ogilvie and Wilson, 1976).

3.1.3. T Cells and Immunity

Tizard and Soltys (1971) reported some degree of cell-mediated immunity to trypanosome antigens in rabbits infected with *T. brucei* or *T. rhodesiense*. However, opposite results were reported by Mansfield and Kreier (1972b) who

found that rabbits infected for 6 weeks with *T. congolense* and then treated with homidium bromide developed Arthus type reaction when challenged with a sonicate of the *T. congolense* used but no delayed-type hypersensitivity and no MIF production.

The cell transfer experiments that have been done are difficult to interpret. Luckins (1972a) injected 10^8 cells mixed from spleen, lymph nodes, and bone marrow of C57B1/6 mice infected with *T. brucei* and treated with Berenil® 3 days later into nonirradiated C57B1/6 recipients. Four hours later, he challenged these recipients with either 500,000 ID₆₃ of the homologous *T. congolense* (group A) or 100,000 ID₆₃ of a heterologous one (group B). The mice of group B died rapidly at the peak of the first wave of parasitemia, whereas those of group A controlled the first parasitemia wave but died at the peak of the second one. No controls of mice reconstituted with unprimed cells or normal unirradiated mice were included. Because of the mixture and number of cells transferred, it is impossible to assign a role to any cell subpopulation. Takayanagi and Nakatake (1976) transferred cortisone-resistant thymocytes from *T. gambiense*-infected and recovered mice into thymectomized and irradiated recipients. Recipient mice showed somewhat higher agglutinin titer on *T. gambiense* challenge and a slight degree of protection. Here again, it is difficult to conclude anything since peripheral T cells of mice of proven immunity were not used. Finally, Gasbarre *et al.* (1980) used an *in vitro* proliferative assay to demonstrate a T-dependent response to *T. brucei*. The response occurred with cells from mice infected for 1 to 2 weeks but disappeared after 3 weeks of infection. The proliferation was also dependent on macrophages.

T-deprived mice, especially congenitally athymic nude mice, have proven very valuable to study the influence of thymus-derived cells on parasite infections (reviewed by Mitchell, 1978). In two reports using thymectomized, X-irradiated, fetal liver reconstituted (CBA × C57B1) F₁ mice infected with *T. brucei*, it was found that the levels of parasitemia were not very different in T-deprived and control animals (Askonas *et al.*, 1979; Faghihi Shirazi *et al.*, 1980). In neither report was the level of residual T cells examined before or after infection, nor the mean time to death reported. However Askonas *et al.* (1979) mention that a proportion of T-deprived and sham-thymectomized mice die earlier than controls, although Faghihi Shirazi *et al.* (1980) write that there was no difference between T-deprived mice and controls.

Jayawardena and Waksman (1977) found that Balb/c nu/nu mice infected with *T. brucei* had a generally lower level of parasitemia than their nu/+ counterparts. Comparative survival data were not reported. In an experiment using 50 Balb/c nu/nu mice and their normal counterparts infected with a strain of *T. congolense*, we found no difference in the prepatent period between the two group of animals (Morrison *et al.*, 1978). At the onset, the mean levels of parasitemia were lower in nude mice but then rose to higher levels than in normal

mice. Very significantly, the mean survival time was 49.5 days for normal mice but only 18.0 days for the nude mice. Similar results concerning both parasitemia levels and survival times have since been obtained with syngeneic nude mice on a C3H and a C57B1/6 background (G. E. Roelants, W. I. Morrison, and K. S. Mayor-Withey, unpublished data). The spleen cell composition of these nude mice after infection showed a twofold increase in B cells, a 22-fold increase in "null" cells, but also a more than tenfold increase in $\text{Ig}^- \text{Thy1}^+$, i.e., T lineage cells (Morrison *et al.*, 1978). The nature of these last cells is not established, but in preliminary experiments, we have found that the spleen cells of *T. congolense*-infected nude mice could be stimulated by T-lymphocyte mitogens and by allogeneic cells (T. W. Pearson and G. E. Roelants, unpublished data). We have also found that Balb/c mice thymectomized at 4 weeks of age and infected about 1 year later by the same strain of *T. congolense* used in the nude mouse experiments survived twice as long as nonthymectomized mice (G. E. Roelants, W. I. Morrison, and K. S. Mayor-Withey, unpublished data). Campbell *et al.* (1978) found a reduced parasitemia and prolonged survival in BALB/c nu/nu mice as compared with nu/+ and thymic reconstituted nu/nu after infection with *T. rhodesiense*. Clayton *et al.* (1979a) infected outbred nu/nu and nu/+ mice with *T. brucei*. The results were difficult to interpret because of the large scatter of parasitemia and survival times, probably because the mice were outbred. Nevertheless, the authors report that in general the first wave of parasitemia is substantially lower in nu/nu than in most nu/+ mice.

In conclusion, it is obvious that the T-cell compartment of lymphocytes has an influence on the course of African trypanosomiasis. This will be even more evident when we analyze immune suppression in trypanosome-infected mice (see Section 3.3). However, the final influence will depend on which T-cell subpopulation is principally activated or inactivated (helper cells, suppressor cells, etc.). Detailed analysis of T cell influence using, for instance, selectively reconstituted nude mice awaits further experimentation.

3.1.4. Increase of Resistance by Immunostimulants

Murray and Morrison (1979a) tried to increase the resistance of A/J and C57B1/6 mice to strains of *T. congolense* and *T. brucei* by injection of *Corynebacterium parvum* or *Bacillus Calmette-Guérin* (BCG). The A/J was shown to be the most susceptible and C57B1/6 the most resistant of a panel of eight inbred strains and three congenic strains used (Morrison *et al.*, 1978). Adjuvant-treated and control mice showed prepatent periods, time to first peak of parasitemia, and levels of first parasitemia that were sometimes statistically significant (at the $p = 0.05$ level) but certainly not striking. *Bordetella pertussis* had no striking effect on those parameters either in mice infected with *T. congolense*. Despite great variations within the groups and a large overlap between adjuvant-

stimulated and control groups, there seems to be an increase in life expectancy in *C. parvum*- and *B. pertusis*-treated mice, whereas BCG appears completely unhelpful.

3.1.5. Conclusions

Immune processes, especially trypanosome strain-specific antibody, are able to protect against homologous challenge. The role of cell-mediated immunity is less well established, and the T-cell subpopulations play a complex role.

The two main impediments to a successful immune response are (1) the wide antigenic diversity and, indeed, antigenic variation of African trypanosomes and (2) the immune depression they induce in infected animals. These two subjects are now reviewed in turn.

3.2. Antigenic Diversity

We have seen that trypanosomes appear to have a high antigenic diversity which makes them “resistant” to immune serum (e.g., Mesnil and Brimont, 1909; Levaditi and McIntosh, 1910). The extent of this diversity and its generation by “antigenic variation” are analyzed.

3.2.1. Antigenic Variation

Doyle (1977) has exhaustively reviewed antigenic variation in man and animals, and we refer the reader to this work for detailed information. A shorter review has also been published recently by Vickerman (1978). What all the evidence shows is that, in naturally and experimentally infected hosts, parasitemia occurs in waves; i.e., a first peak of parasitemia is brought under control, probably by the immune system, but is followed by another peak which is brought under control as well, and the succession of peaks and troughs of parasitemia continues throughout the disease.

It is important that successive waves are constituted of trypanosomes that are antigenically different (Gray, 1962, 1965, 1966, 1970; Wilson and Cunningham, 1972). When trypanosomes are isolated from several animals, even in the same area, at the same time or over a period of time, most trypanosome populations are also antigenically different. For instance, Dar *et al.* (1973) obtained 73 specific antisera to study 23 primary isolates of *T. vivax* in the Masai-Mara and Lugala areas. They found some inter- and intraarea cross reactions, but all the parasite populations were essentially different. There is some serologic evidence that identical or similar antigenic variants may appear in two different parasitemia peaks in the same animal (Wilson and Cunningham, 1971; Nantulya *et al.*, 1979). Antigenic variation is rapid: Jones and Clarkson

(1974) reported the appearance of at least four serologically recognizable variants within 8 days of the first appearance of *T. vivax* in animals exposed to *G. morsitans* bites. The variant types appearing in various hosts infected with the same organism may be different. De Gee *et al.* (1979) found that the same clone of *T. vivax* adaptable to mice (Leefflang *et al.*, 1976) leads to a series of different variants whether injected into goats or mice. Within the same host, it appears that at any given time bloodstream trypanosomes are antigenically different from tissue ones and that for *T. brucei* in rats, new variants appear first in the lymph nodes (Tanner *et al.*, 1980). Monomorphic slender forms of *T. brucei* have indeed been shown to be flushed from the lymphatic system into the bloodstream (Ssenyonga and Adams, 1975).

At this point, it is worth while to make a technical digression. As very well pointed out in Doyle's review (1977), most studies on antigenic variation have been done with "strains" of parasites which inevitably constitute many different antigenic types. Moreover, variant antigenic types are most often defined by serologic means, i.e., with antisera from infected animals which will certainly contain antibodies of many different specificities (hence the somewhat confusing results reported in the literature). Further studies on antigenic variation imperatively have to be done with defined clones of trypanosomes (Lumsden *et al.*, 1967). Furthermore, it is obvious but sometimes overlooked that assays should be used that detect surface antigenic differences such as agglutination, neutralization of infectivity (Lumsden *et al.*, 1973), or immunofluorescence on live or formalin-fixed trypanosomes (Nantulya and Doyle, 1977) and not assays detecting common internal trypanosome antigens such as immunofluorescence on acetone- and ethanol-fixed cells or microplate-linked immunosorbent assays (Luckins, 1977). The growth *in vitro* of infective forms of *T. brucei* (Hirumi *et al.*, 1977a,b; Tanner, 1980) and *T. rhodesiense* (Hill *et al.*, 1977; Bienen *et al.*, 1980; Hill, 1980) offers an ideal means to study mechanisms of antigenic variation and has already permitted the demonstration that antibodies are not necessary for its induction (Hirumi *et al.*, 1980; Doyle *et al.*, 1980).

3.2.2. Biochemistry of the Variant Antigens

It was first reported that variant specific trypanosomal antigens were almost completely restricted to soluble cytoplasmic proteins (Brown and Williamson, 1962). However, it has become quite evident that although variant antigens are associated with other subcellular fractions (Rovis and Baekkeskov, 1980), the immunologically significant variant antigenic determinants are borne on a unique glycoprotein forming an external coat completely surrounding the trypanosome bloodstream form, including its flagellum (Vickerman, 1969; Vickerman and Luckins, 1969; Allsopp *et al.*, 1971; Allsopp and Njogu, 1974).

Some characterization of common and variant antigens of *T. brucei* gam-

biense was achieved by immunoelectrophoresis (Afchain *et al.*, 1975), but most of our knowledge comes from the work of Cross and co-workers on *T. brucei brucei* (Cross, 1975, 1978; Bridgen *et al.*, 1976). They showed that clone-specific antigens constituting the surface coat are unique glycoproteins of mol. wt. about 65,000, having about 600 amino acids and 20 monosaccharide residues. Using four clones of trypanosomes isolated from a rabbit over a period of 3 weeks after injection of a stock of *T. brucei*, they showed that there were complete differences in the sequence of the first 20, 35, 34, and 34 N-terminal amino acids, respectively. These glycoproteins appear to be purely structural, as no evidence could be obtained for an enzymatic function. However, there is good evidence that isolated variant antigen can "consume" complement (Musoke and Barbet, 1977). Studies *in vitro* showed that *T. brucei* incorporates [³⁵S]methionine into the variant antigen but that the rate of synthesis was only about 8% of that *in vivo* (Taylor and Cross, 1977). Using a clone of *T. congolense*, Rovis *et al.* (1978) showed that this species also has a single specific glycoprotein as surface antigen. We do not know of similar biochemical studies reported for other species of African trypanosomes.

Brett and Voorheis (1980) have reported that the elongation of the sugar residues takes place while the glycosyl receptor is attached to the polypeptide, only when the polypeptide resides in the plasma membrane, and using nucleotide sugar donors from the cytoplasmic side. Rovis and co-workers, however, using *T. brucei* grown in the presence of tunicamycin, a specific inhibitor of asparagine glycosylation, have shown conclusively that a first step of glycosylation occurs concomitantly with the synthesis of protein followed by a second step taking place after translation and that some of the glycosylation follows the dolichol cycle (Rovis and Dube, 1981a,b).

Glycopeptides may constitute some cross-reacting determinants of variant specific surface antigens (Barbet and McGuire, 1978). However, they seem to elicit antibodies only in some rabbits and only if the biochemically isolated surface protein is used as immunogen. Moreover, these determinants are located on the C-terminal end of the protein, that is, the part near the plasma membrane, where they are masked from the environment and not available for binding by antibody (Cross, 1979a,b). Thus, these antigens do not appear to be relevant to the immunogenicity of the variant antigen on the intact trypanosome or to be useful to induce protective immunity. In this regard, it should be pointed out that the variant coat is very dense and appears to be mainly in an α -helix (L. Rovis, personal communication), so that despite the great overall immunogenicity of the whole variant glycoprotein, only few determinants on the N-terminal end may be available for interaction with the immune system. One should thus think of exposed parts of variant antigens as sheets of highly repetitive determinants located close to each other rather than multiple different determinants along the whole glycoprotein. This may have implications for the polyclonal activation or

immunosuppression which is analyzed later. In this regard, it should be noted that monoclonal antibodies raised against isolated *T. brucei* variant antigen in Balb/c mice bind most often to acetone-fixed but not to live or formalin-fixed trypanosomes (Pearson *et al.*, 1981).

The fluidity of the variant glycoprotein was examined by similitude with other systems (reviewed in Loor, 1980). Giannini and D'Alesandro (1978) reported that capping of the surface coat of *T. lewisi* grown in rats could be induced at either 0°C or 37°C with rabbit-anti-rat immunoglobulin but not with Fab' fragments. Barry (1979), however, reported that the capping of *T. brucei* surface antigens towards the flagellar pocket region occurred at 37°C but not at 0–4°C and that it removed all variant antigen and revealed common antigen on the cell membrane. We have personally observed that capping of *T. congolense* surface coat was very dependent on the strength of the reagent used to induce it. Early antibodies induced rapid capping even when all manipulations were performed strictly at 0°C. Antibodies from hyperimmune serum, however, appeared to cross link the antigens in such a way that no capping occurred even at 37°C. In our experiments, capping, when it occurred, was not complete but left variant antigen at the surface of trypanosomes. Variant antigen-antibody complexes rapidly attached to macrophages. Some complexes were interiorized, and some remained exposed at the macrophages surface for at least 24 hr (G. E. Roelants and L. Rovis, unpublished observations). Barry (1979) also observed that the same antigen type reappeared after capping and that antibody-induced capping was probably not the mechanism of induction of antigenic variation.

3.2.3. Molecular Genetics of Antigenic Variation

Antigenic variation when confined to laboratory animals using cloned antigenically defined trypanosomes is a sequential expression of a series of membrane surface proteins. The initial waves of parasitemia consist of trypanosomes uniformly expressing a single membrane antigen. After the third or fourth wave, the populations are generally heterogeneous, with several or many antigenic types present, but each cell nonetheless expressing only a single surface antigen. The largest number of antigenic types originating from a clone is 101, reported for the species *T. equiperdum* (Capbern *et al.*, 1977). There is no definitive order of antigen appearance on the trypanosome surface in the different waves of parasitemia, but they do appear in a sequence that can be predicted statistically. Antigens that have previously appeared in an infection can reappear, indicating that the genes for the antigens are stably inherited (Doyle, 1977). The appearance of new antigens in a normal infection as well as in tissue culture is consistent with the suggestion that a single trypanosome changes its antigen at a very low frequency rather than a number of trypanosomes switching antigens in concert (Doyle *et al.*, 1980).

The number of antigens potentially capable of being expressed from a clone is not known but is likely to be at least in the hundreds. The large antigen diversity and the mechanism by which trypanosomes change expression from one antigen to another have drawn the interest of the molecular biologists to trypanosomiasis. With the advent of recombinant DNA technology, tools have become available to study the molecular basis of antigenic variation. The experimental details of recent studies on the molecular biology of antigenic variation have been reviewed and discussed in detail by Marcu and Williams (1981). We present here only a summary of the pertinent models and conclusions.

Initial studies showed that the messenger RNAs (mRNAs) coding for the variant antigens in the cytoplasm of the trypanosome were abundant RNA sequences and that the abundant class of RNA sequences was unique to each cell clone expressing a different variant antigen in *T. brucei* (Williams *et al.*, 1978) and *T. gambiense* (Meritt, 1980). These studies suggest that the trypanosomes are highly specialized cells structured to produce very large quantities of the membrane surface antigen and lay the ground for the cloning of complementary DNA in bacteria.

The first recombinant DNA studies of the variant antigen gene system in trypanosomes were reported by Williams *et al.* (1979) and Hoeijmakers *et al.* (1980). Their approaches to the growth and isolation of complementary DNA have taken advantage of the presence of the unique mRNA sequences in the trypanosome cytoplasm (Alwine *et al.*, 1977). Complementary DNA was copied from mRNA coding for variant antigens and subsequently cloned and grown in the bacteria, *E. coli*. Hybridization studies in these two reports showed an unusual instability of the DNA structure surrounding several of the variant antigen genes. Hoeijmakers *et al.* (1980) proposed a model that seemed to fit their data describing DNA rearrangements observed during the expression of the variant antigens. Simply, they proposed that in order for a variant antigen to be expressed, the gene coding for that specific antigen is first duplicated from a basic library copy in the DNA in a manner similar to nuclear DNA duplication prior to cell division. Following expression of this extra gene copy, it is lost in some undescribed manner. They termed the extra gene an "expression-linked extra copy" or ELC.

Such a novel mechanism explaining gene expression has been described previously for the phenotypic expression of different mating types in yeast (Hicks *et al.*, 1977). The appearance of an extra antigen gene copy during its expression has also been briefly reported by Agabian *et al.* (1980). Very interestingly, they also reported on a comparison of variant antigen gene rearrangements with the arrangement of the tubulin genes in the DNA of trypanosomes. Tubulin genes appear stably inherited in different cell clones, leaving the instability and rearrangements of DNA apparently restricted to the variant antigen genes. No specific model was presented by Williams *et al.* (1979) to explain

variant antigen expression. Although an "expression-linked extra copy" model may perhaps explain some of the genetic data, it is clear from recent results (Williams *et al.*, 1980; Donelson *et al.*, 1981) that there are other phenomena involving the variant antigen genes that certainly complicate such a model if not invoke other mechanisms of antigen gene expression. For example, they observe no specific arrangement of DNA structure that can be correlated with expression of a single variant antigen. Indeed, there are dissimilar DNA structures with respect to the antigen gene between two separate cell clones expressing the same antigen. It is also possible that some of the instability demonstrated in the DNA surrounding the antigen genes may represent an evolutionary "hot spot," creating mutational changes that contribute indirectly to antigen diversity.

In addition to the discrepancy in the data supporting and opposing the ELC model, there is a difference in the purported antigen gene copy number in each of the two studies. Hoeijmakers *et al.* (1980) find multiple copies of the variant antigen gene during the time the gene is expressed. Williams *et al.* (1980, 1981) and Donelson *et al.* (1981) have described the presence of multiple gene copies present in both the expressed and the nonexpressed states. The number of antigen genes is different for each of the genes examined, varying between two and four, but the number remains constant in both the expressed and nonexpressed states. This is contrary to the results presented by Borst *et al.* (1981), yet they do mention the possibility of a polyploid organism. The multiplicity of variant antigen genes suggests the possibility of a diploid organism.

The question of sexual processes existing in trypanosomes has been an open question that has received sporadic attention for years (Walker, 1964). The existence of sexual recombination would greatly potentiate the basic number of antigens capable of being expressed in an infection and might suggest methods to determine the size of the antigen gene pool. Only recently, however, have solid data appeared that argue in favor of a sexual cycle. Tait (1980) has described an isoenzyme analysis of 19 enzymes on 17 separate trypanosome stocks. The study provides strong evidence for the occurrence of a sexual cycle in trypanosomes. Eight of the enzymes studied showed electrophoretic variation in patterns consistent with multiple enzyme alleles that would be expected in a diploid organism. Indeed, the different enzyme patterns could be explained by recombination of alleles making heterozygotes for some of isoenzymes described. In a similar study, Gibson *et al.* (1980) have shown the same phenomena for a few of the same enzymes studied by Tait. They discuss briefly the possibility of sexual recombination, presumably because allelic type isoenzymes patterns were observed in a minority of the enzymes studied.

If there is a sexual cycle in the trypanosomes, the potential number of variant antigens is indeed very large. Recombination would demonstrably increase the potential number of antigens capable of being expressed from a single trypanosome. It would also raise some question as to the validity of the familial

type designations such as "serodeme" and "zymodeme," terms used to describe relatedness in variant antigen repertoires and isoenzyme repertoires. The central question to which most of the molecular studies are directed is still unanswered. What is the size of the variant antigen gene pool? The question, we hope, will have an answer soon.

3.2.4. Cyclic Transmission and Antigenic Variation

The variant antigen coat is rapidly lost when trypanosomes transform from bloodstream form to fly forms either *in vitro* or *in vivo* (Barry, 1977). In the fly, the midgut forms are devoid of coat; *T. brucei* and *T. congolense* metacyclic forms in the salivary gland regain a coat but not *T. vivax*. Jenni, Nantulya, and Doyle, in a series of experiments, studied in detail the antigenic types of both metacyclic forms of *T. brucei* (Jenni, 1977a,b) and *T. congolense* (Nantulya *et al.*, 1980a,b) and variants appearing in the host after tsetse fly bite. The main conclusions of these studies are that the antigenic diversity of the variant antigen is restricted within a given fly but that on injection of the trypanosomes into susceptible host, metacyclic variants transform rapidly into bloodstream form variants which are antigenically different and are also more heterogeneous. Even when the fly has been infected with a clone, resulting parasites are heterogeneous and different from the initial clone used to infect the fly (Nantulya *et al.*, 1980a). Some degree of antimetacyclic immunity can be demonstrated after fly bite and Berenil® treatment, but it is short lived (Nantulya *et al.*, 1980b). In contrast to this work, it has also been reported that not only does a rapid heterogeneity occur when metacyclic variants are injected in the host (Le Ray *et al.*, 1977) but that the metacyclic variants themselves have antigenic diversity within a given fly (Le Ray *et al.*, 1978; Barry *et al.*, 1979).

Other workers have concluded as Jenni, Nantulya, and Doyle that variant antigen heterogeneity does not extend to the metacyclic population but comes about immediately on transmission (Hudson *et al.*, 1980). Finally, Jones and Clarkson (1972a,b) as well as De Gee *et al.* (1976) showed a large degree of antigenic variation after fly passage of *T. vivax*.

It is worth mentioning that one fact that complicates even more an already complex picture is that different antigenic types are able to compete with each other and that once one type is established it may impair the emergence of other ones (Herbert, 1975; Seed, 1978). From a series of experiments, Gray (1962, 1965, 1966, 1975) concluded that some antigenic types appeared to emerge more often than others after tsetse fly passage, and these have been called "predominant" antigens. Some antigens are even more predominant than others and appear to be detected with high frequency early after tsetse fly bite; these were designated "basic" antigens. Unfortunately, these studies relied on strains of trypanosomes, not on clones, and the heterogeneity of the populations studied is

difficult to evaluate since “basic” or “predominant” antigens were never isolated but detected serologically with serum of infected animals containing, as discussed earlier, mixtures of antibodies. If strain-basic antigens exist, it would be predicted that a certain degree of immunity would develop, at least for animals kept in the same area. As we have seen before from other authors and from Gray himself (1970), this is unfortunately not the case. Kosinski (1980), however, in a computer analysis of antigenic variation data from the literature found a strong tendency towards a reproducible order of variants. He concluded that random generation and selection by growth rate alone could not produce the degree of variant orderliness reported in the literature, but again most workers were not using clones.

3.3. Polyclonal Activation and Immunodepression

In 1972, Goodwin and colleagues reflected on the high susceptibility to certain diseases, mainly infections, and, on the other hand, the diminution of autoimmune diseases in sleeping sickness patients. They reported that in mice infected with *T. brucei* there was a decrease in the agglutinin response to sheep erythrocytes, especially after 2 weeks of infection. *Trypanosoma brucei*-infected rabbits all had increased agglutinin titers even before immunization with sheep erythrocytes, and the titers did not increase further after challenge. Since then, a large amount of data has been accumulated to show that trypanosomes cause polyclonal activation of B cells leading to elevated levels of immunoglobulin of various specificities unrelated to trypanosome antigens which leads to B cell exhaustion; moreover, trypanosomes induce depression of T and B cell responses by a mechanism that involves some kind of T suppressor cell and macrophages.

3.3.1. Elevated Levels of Immunoglobulin

Gidel (1962) and Bideau *et al.* (1966) reported elevated IgM in cattle exposed to tsetse flies in enzootic areas. Since then, many reports have appeared on increases in IgM (two- to tenfold) and later in IgG (twofold) in trypanosome-infected cattle (Luckins, 1972b, 1976; Clarkson and Penhale, 1973; Clarkson *et al.*, 1975a,b; Clarkson, 1976; Luckins and Mehltitz, 1976). Levels of Ig returned to normal 2 to 3 weeks after treatment with Berenil®. There is also increased serum Ig in human patients suffering from trypanosomiasis or also from malaria; this is mainly IgM, which contains antiparasite antibody, heterologous antibody, and autoantibody (Greenwood, 1974a; Mattern *et al.*, 1978). In an accidental laboratory infection with trypanosomes of a defined stock of *T. brucei*, increased IgM and IgE levels were found which were reported to be specific for trypanosome antigens (Herbert *et al.*, 1980).

High IgM levels including heterologous and autoantibodies were also found in the monkey *Macaca mulatta* infected with *T. brucei rhodesiense*, *T. brucei*

gambiense, and *T. brucei brucei* (Houba *et al.*, 1969), in *T. brucei brucei*-infected mice (Hudson *et al.*, 1976; Corsini *et al.*, 1977; Mitry-Martin *et al.*, 1979), and in acquired and experimentally induced trypanosomiasis of sheep (P. K. I. Mackenzie *et al.*, 1979).

Polyclonal activation and high Ig levels are accompanied in cattle by gross changes in the lymphoid organs with a high proliferative response, especially in the paracortex, with germinal center formation and appearance of large numbers of plasma cells with internal IgM (reviewed in Morrison and Murray, 1979a; Murray *et al.*, 1980; Masake and Morrison, 1981). In the infected mouse spleen, one sees a transient increase of T cells and large increases and proliferations of B and null cells (Morrison *et al.*, 1978; Mayor-Withey *et al.* 1978). These increases are different in strains of mice of different susceptibility to trypanosomes, and their amplitudes seem to be correlated with higher sensitivity (Morrison *et al.*, 1978).

3.3.2. Immunodepression

Numerous studies have shown that trypanosome-infected mice have an impaired antibody response to antigens such as sheep erythrocytes (Murray *et al.*, 1973, 1974a,b; Longstaffe *et al.*, 1973; Hudson *et al.*, 1976; Mansfield and Bagasra, 1978; Hudson and Terry, 1979; Assoku *et al.*, 1979), TNP on a carrier protein (Hudson *et al.*, 1976; Mansfield and Bagasra, 1978), polyvinyl pyrrolidone (Mansfield and Bagasra, 1978), SIII polysaccharide (Hudson *et al.*, 1976; Mansfield and Bagasra, 1978; Assoku *et al.*, 1979), and loupin-ill and lymphocytic choriomeningitis virus (Reid *et al.*, 1979). Skin responses to oxazolone are somewhat diminished but still rather near normal until terminal stages of the infection (Urquhart *et al.*, 1972, 1973; Murray *et al.*, 1974b). Trypanosome-infected mice also present a delay in allogeneic skin graft rejections, the delay increasing when grafts are implanted after prolonged periods of infection (Pearson *et al.*, 1978). In experiments in which it was tested, immune competence was rapidly restored after treatment with a trypanocidal drug.

In rats, it was found that *T. brucei*-infected animals challenged with *Nippostrongylus brasiliensis* could not expell the worms, could not provide passive protection by their serum, did not produce reagenic antibody, and did not have any increase in their intestinal mast cells (Urquhart *et al.*, 1973). In the field vole, *Microtus montanus*, immunodepression was shown for both primary and secondary responses to sheep erythrocytes and bovine serum albumin after infection with *T. brucei gambiense* (Ackerman and Seed, 1976c). These responses returned to normal 3 days after treatment with Suramin®.

Considering that immunodepression in cattle could have considerable implications for the success of vaccination campaigns in Africa, the response of cattle to a variety of infectious agents and vaccines has been studied by several

investigators. Holmes *et al.* (1974) found that the antibody levels of ongoing primary and secondary responses to clostridial vaccine were lowered by infection with *T. congolense*. Scott *et al.* (1977) treated Zebu cattle with foot-and-mouth disease vaccine alone or in combination with clostridial vaccine and exposed the cattle with or without Berenil® treatment in a tsetse fly area where *T. congolense* was the main challenge. The animals not receiving Berenil® had 0.3–0.9 log₁₀ units lower circulating antibodies than did a control group protected by Berenil®. The authors concluded that “despite the evidence of distinct immunosuppression in cattle from trypanosome endemic areas, it was unlikely to impede the development of protection against foot-and-mouth disease and clostridial infection by vaccination” on the basis that infected animals still had titers of antibody that would generally be considered as protective. However, the degree of protection of these animals was not actually tested. Moreover, the cattle were exposed to possible tsetse fly bites for only 10 weeks, and another group of animals receiving both vaccines in a tsetse fly-free area but infected experimentally with *T. congolense* 14 days prior to vaccinations showed that 31 days later four out of six animals had antibody levels below those necessary to give 95% protection to needle challenge. For these reasons, the conclusion of no interference of trypanosomiasis on vaccination appears rather sweeping.

Rurangirwa *et al.* (1978) found that cattle that had recovered from bovine pleuropneumonia gave a weaker secondary response than controls when challenged with *Mycoplasma mycoides* 18 days after infection with either *T. congolense* or *T. vivax*. *Trypanosoma congolense* and *T. vivax* also suppressed humoral responses to *Leptospira biflexa* and *Brucella abortus* (Rurangirwa *et al.*, 1979, 1980b), the suppression being entirely reverted for the former and partially for the latter by Berenil® treatment, and, finally, to rinderpest virus vaccine (Rurangirwa *et al.*, 1980a).

In contrast to these examples of immunodepression, Sollod and Frank (1979) reported only insignificant differences in the immune responses of cattle infected with *T. congolense* to DNP-ovalbumin bovine parainfluenza 3 and a wild *E. coli* bacteriophage. The experiments were performed during the first 5 weeks of infection, and immunodepression might take longer to be established in cattle. Finally, De Gee and Shah (1979) reported that superinfection with *T. brucei* in calves already carrying *T. vivax* for 30 days resulted in a weaker anti-*T. brucei* response than in control animals. Depression of antitrypanosome responses in *T. brucei*-infected mice were also reported by Sacks and Askonas (1981). In addition, they presented evidence that trypanosome-induced suppression of the antiparasite response and IgM in particular determines the course of infection by trypanosomes.

In man, immunosuppression was shown in a group of 38 *T. brucei gambiense* patients in Nigeria compared to 43 controls of the same ethnic group, age, and sex (Greenwood *et al.* 1973; Greenwood, 1974b). The sleeping sickness

group had a marginally lower skin response to PPD ($p = 0.02$), a lower response to *Candida* antigen ($p < 0.01$), but identical responses to streptococcal antigen. Forty-two percent of the patients had a skin reaction to dinitrochlorobenzene compared to 81% of the controls. Finally, there was a significantly lower ($p < 0.01$) humoral response to the H antigen of *Salmonella typhi* but a similar response to the O antigen.

Two more species were tested. Mansfield and Wallace (1974) found that New Zealand white rabbits infected with *T. congolense* and immunized 3 weeks later with mycobacterial protein show, still 3 weeks later, a much milder skin test response to PPD, a depressed lymphocyte stimulation by PPD *in vitro*, and no MIF production. Mackenzie *et al.* (1975) reported that trypanosome-infected sheep often succumb to bacterial pneumonia and that sheep injected with *Vibrio foetus* antigen 7 and 21 days after *T. congolense* infection develop a tenfold lower secondary agglutinin response.

Another phenomenon reported in connection with immunodepression is the susceptibility of *T. brucei gambiense*-infected field voles, *Microtus montanus*, to Ehrlich's tumor transplants originating from albino mice, although normal animals usually reject them, a suppression attributed by the authors to indole-3-ethanol which appears to be synthesized by *T. brucei gambiense* (Ackerman and Seed, 1976a,b). It was also reported that rheumatoid arthritis and other autoimmune diseases are uncommon in Nigeria, that *T. brucei brucei* and *T. congolense* suppressed experimental allergic neuritis in some but not all rabbits (Allt *et al.*, 1971; Mansfield and Kreier, 1972a), and that *T. brucei brucei*-infected rats were less susceptible to experimental allergic diseases (A. R. Mackenzie *et al.*, 1979).

3.3.3. Mechanisms of Immunodepression

The mechanism by which trypanosomes induce immunodepression has been analyzed at the cellular level by using mitogens specific for either T or B cells, by investigating the stimulation *in vitro* by antigens, and by ascertaining the inhibitory activity of various cell subpopulations from trypanosome-infected animals. Most of these studies have been done in mice.

Stimulation of lymphocytes *in vitro* by *E. coli* lipopolysaccharide (LPS), a B-cell mitogen in mice, is markedly diminished in trypanosome-infected animals whether ascertained by thymidine or thymidine analogue incorporation into DNA (Jayawardena and Waksman, 1977; Pearson *et al.*, 1978) or by analysis of the absolute number of cells in culture (Corsini *et al.*, 1977). Reactivity of T cells to the mitogens Con A (Jayawardena and Waksman, 1977) and in a mixed lymphocyte culture across the major histocompatibility barrier (Pearson *et al.*, 1978; Askonas *et al.*, 1979) as well as the generation of cytotoxic T cells (Pearson *et al.*, 1978) were also depressed after infection by either *T. brucei* or *T. congolense*. Memory T and B cells are equally affected (Askonas *et al.*, 1979).

Equally depressed are primary responses to the thymus-dependent antigen sheep erythrocytes (Eardley and Jayawardena, 1977; Wellhausen and Mansfield, 1979) and the thymus-independent antigen DNP-ficoll (Eardley and Jayawardena, 1977; Askonas *et al.*, 1979). Thus, it appears that in mouse spleens most lymphoid cell activities are impaired after trypanosomal infections. It was shown that the diminished reactivity was not a result of dilution of relevant cells by large numbers of null cells which appear in the spleen of trypanosome-infected mice (Roelants *et al.*, 1979a). It was demonstrated that the immunodepression is caused by active suppression; cells from infected mice inhibit stimulation of normal cells by antigen (Eardley and Jayawardena, 1977; Wellhausen and Mansfield, 1979) or by lectins or allogeneic lymphocytes (Pearson *et al.*, 1979).

The nature of the suppressor cells is still a matter of debate, but it is clear that the mechanism involves T cells and macrophages. One school of thought holds that suppression is mediated by suppressor T cells which are sensitive to anti-Thy 1 and complement, are present in the effluent of nylon wool columns, are insensitive to antilymphocytic serum, are short lived, i.e., lost 4 weeks after thymectomy, and bear the Ly 1, 2, and 3 antigens (Jayawardena *et al.*, 1978). The opposite view is that suppressor T cells are not involved and that all the suppressor activity is caused by macrophages (Wellhausen and Mansfield, 1979; Askonas *et al.*, 1981). However, if the suppressor activity were entirely caused by suppressor macrophages, it would be relieved by indomethacin treatment *in vitro* (Kempt *et al.*, 1981); this was not found to be the case (Wellhausen and Mansfield, 1980b). Two laboratories have found that anti-Thy 1 and complement treatment or macrophage depletion of cell suspension from infected mice both alleviate suppression but not completely (Corsini *et al.*, 1977; Pearson *et al.*, 1979). Gasbarre *et al.* (1981a) also showed that both T-cell and macrophage populations are affected by the parasite infection.

Part of the confusion may come from the fact that usual techniques of cell fractionation do not give similar results with spleen cells from normal and trypanosome-infected mice. Thus, it was found that with infected mice anti-Thy 1 and complement treatment killed only about 50% of Thy1⁺ cells, that activated macrophages were largely nonadherent, although they were removed by carbonyl iron, and that most small T lymphocytes but not T blasts adhered to nylon (Pearson *et al.*, 1979). It is thus imperative to determine in each experiment the actual phenotype of the cells recovered from these manipulations rather than to rely on data obtained with normal cells. In this connection, it should be emphasized that "null" cells (Ig⁻Thy1⁻) present in very large numbers in the spleens of trypanosome-infected mice (Roelants *et al.*, 1979b; Mayor-Withey *et al.*, 1978) are not the suppressor cells. Indeed, they belong to the erythroid series and are mainly normoblasts as determined by electron microscopy (W. I. Morrison and E. Weiss, personal communication) and by the use of a specific monoclonal antibody donated by C. Milstein (G. E. Roelants, unpublished observation).

Another controversy bears on the respective roles of suppression, whatever the actual mechanism is, and B-cell "exhaustion" resulting from polyclonal activation. Here, one school of thought holds that suppression is important early in infection and that there is an intrinsic defect in B cells later on (Corsini *et al.*, 1977). These conclusions were reached from experiments (1) in which mice were surviving only for 19 to 30 days (one may wonder what early and late events really mean in such acute infections) and (2) performed on athymic nu/nu mice (Clayton *et al.*, 1979a) on the false assumption, amply documented now, that nude mice are deprived of T-lineage cells (Loor and Roelants, 1974, 1975; Scheid *et al.*, 1975; Roelants *et al.*, 1975, 1976; Loor *et al.*, 1975, 1976; Sato *et al.*, 1976; Roelants and Mayor-Withey, 1977) or that on trypanosomal infection these cells do not mature to functional T cells (see Section 3.1.3). Mansfield and Bagasra (1978), on the other hand, hold that active suppression is the main cause of immunodepression and that there is no intrinsic defect of B cells until mice are in terminal stages of infection. Pearson *et al.* (1979) have calculated that complete suppression *in vitro* was still achieved at ratios of cells from infected mice to potential reacting cells from normal mice of 1 to 250 for either T lymphocytes or macrophages.

Immune unresponsiveness is rapidly alleviated after treatment with trypanocidal drugs. Thus, it was found that responses to LPS, Con A, and allogeneic cells returned to normal 5 to 8 days after treatment despite the fact that neither spleen cell composition, analyzed on cells in suspension, nor the architecture of the spleen, analyzed on histological sections, had returned to normal (Roelants *et al.*, 1979a).

It was shown that the degree of susceptibility to *T. congolense* of various inbred strains of mice is directly correlated with the time of onset of immunosuppression and its intensity (Pearson *et al.*, 1982). A very important observation is that only spleen cells are suppressed and are actively suppressive in both *T. brucei rhodesiense* (Wellhausen and Mansfield, 1979) and *T. congolense* (Kar *et al.*, 1981) infections and that lymph nodes or peripheral blood lymphocytes are not. This observation extends to *T. brucei* which, unlike *T. congolense*, also invades tissues including lymph nodes (Kar *et al.*, 1982). Freeman *et al.* (1974) assumed that antigen-binding and reactive cells were not depleted in trypanosome-infected mice because soon after treatment they would give normal levels of plaque-forming cells on challenge with sheep erythrocytes. However, it was found that no rosette-forming cells to sheep erythrocytes could be detected in *T. congolense*-infected mice before or after challenge with sheep erythrocytes (J. London, personal communication).

The type of approach described for mice has been rarely used on man and cattle. It was reported that monkey peripheral blood lymphocytes infected with *T. rhodesiense* have diminished or abolished reactivities to T-cell mitogens and to allogeneic lymphocytes (Dallas, 1980). Sollod and Frank (1979) did not detect a difference between *T. congolense*-infected and noninfected cattle in the pe-

ripheral blood lymphocyte response to PHA, PWM, and allogeneic cells. Masake *et al.* (1981) observed a diminution in the stimulation index of peripheral blood lymphocytes from Boran cattle infected with *T. congolense*. However, the diminution was caused by a higher background DNA synthesis in the cells from infected animals. This high background reverted to normal level with treatment with Berenil®. There is also a vague indication that bovine bone marrow granulocyte/macrophage but not erythroid colony formation is inhibited by serum of infected animals (Kaaya *et al.*, 1979).

It has been claimed that trypanosome antigen and “substances” of trypanosome-infected animals have either mitogenic or suppressive effects on lymphocytes (e.g., Esuruoso, 1976; Assoku and Tizard, 1978; Moulton and Coleman, 1979), but the effects reported are quite minute, unconvincing, and unreproducible (Corsini *et al.*, 1977; Mansfield *et al.*, 1976; Clayton *et al.*, 1979a). Lately, it has been claimed that membrane fractions of trypanosomes mimic the immunosuppressive activity and mitogenic effects of living parasites (Clayton *et al.*, 1979b) and that the intrinsic immunosuppressive activity of different trypanosome strains varies with parasite virulence (Sacks *et al.*, 1980). However, the effect is quite dose dependent, as it appears for the injection of membrane fractions derived from 10^8 but not 10^7 parasites, acts only on the IgM and not the IgG response, and the experiments were conducted only on one type of F_1 specific pathogen-free hybrid mice. In this regard, it is interesting that a clone of *T. brucei* that kills C3H mice in 7 to 10 days and grows up to 10^9 trypanosomes per ml does not suppress mitogenic responses (S. K. Kar, personal communication) and that CBA/N mice which are defective in B cell maturation and IgM production survive better than conventional mice after *T. brucei* challenge (Gasbarre *et al.*, 1981b).

Finally, we want to add that the mechanisms of cellular suppression are not the only ones involved in immunodepression, since accelerated levels of Ig catabolism (Murray, 1974) and a drop in levels of complement (Rurangirwa *et al.*, 1980c) have been shown in trypanosome-infected animals. Moreover, as mentioned earlier, biochemically isolated variant-specific surface antigen of *T. brucei brucei* does activate complement (Musoke and Barbet, 1977; A. C. Allison, personal communication).

4. GENETIC BASIS OF SUSCEPTIBILITY TO AFRICAN TRYPANOSOMIASIS

4.1. Experimental Evidence in Mice

Much evidence has been provided in recent years for the genetic control of susceptibility to disease (reviewed in Lilly and Pincus, 1973; McDevitt *et al.*, 1974; Bluestein, 1977; Ryder and Svejgaard, 1977).

In a series of experiments, Morrison and colleagues (1978, 1979; Morrison

and Murray, 1979b) have studied the susceptibility of inbred and congenic strains of mice as well as their F_1 hybrids and F_1 -parental back crosses to various strains of *T. congolense*. They found that there is a rank in the level of susceptibility among the various inbred strains as measured by mean survival time ranging from about 16 days for A/J mice to 110 days for C57B1/6 mice, six other strains being intermediate: SWR, 17 days; 129, 23 days; DBA/1, 36 days; Balb/c, 50 days; C3H/H1, 59 days; AKR, 82 days. The F_1 hybrids between C57B1/6 and A/J had a mean survival time that was somewhat higher than that of the C57B1/6 mice themselves, 133 days, whereas (C57B1/6 $\times$ C3H/He) F_1 survived only about 90 days. There was no difference in the prepatent period among the various strains and F_1 hybrids studied. A/J, SWR, 129, and Balb/c mice had similar levels of parasites at the first peaks of parasitemia, about 10^8 /ml, despite differences of survival time ranging from 16 to 50 days. Likewise, DBA/1, C3H/He, C57B1/6, and (A/J $\times$ C57B1/6) F_1 have similar levels of first parasitemia about 5×10^6 /ml, despite survival times as different as 36 and 133 days. Finally, AKR has consistently very low levels of first parasitemia, about 10^4 /ml, but nevertheless dies faster than C57B1/6 or its F_1 hybrids. Moreover, in extended experiments it was shown that (A/J $\times$ C57B1/6) F_1 hybrids, which survive consistently better than their C57B1/6 parent, also consistently show higher levels of parasitemia. Likewise, back crosses of B6A/ F_1 to A/J all had a similar high initial peak of parasitemia although they showed a range of survival times going all the way from that of A/J to that of C57B1/6 mice. Thus, despite claims to the contrary (Murray and Morrison, 1979b), there is clearly no direct correlation between the peak levels of the first wave of parasitemia and susceptibility.

Studies of C57B1/10 Sn ($H2^b$) mice and its congenic counterparts for the major histocompatibility region B10A/SSn ($H2^a$), B10BR/SgSn ($H2^k$), as well as C57B1/10 and the congenic B10D2/n ($H2^d$) showed that the $H2$ allotype had no or little influence on susceptibility. Finally, segregation studies on F_1 hybrids and parental back crosses showed that although resistance was generally dominant, the degree of resistance is under polygenic control. Others have also reported differences of susceptibility of mice to other strains of *T. congolense* (Jennings *et al.*, 1978; Whitelaw *et al.*, 1980) or *T. brucei* (Selkirk and Sacks, 1981), but no crosses or back crosses were analyzed, and it is not known if the differences were under genetic control. There is an intriguing possibility that the differences in susceptibility, particularly the long survival of C57B1, could be a result of these mice having a dominant contrasuppressor circuit (Gershon *et al.*, 1981; Yamauchi *et al.*, 1981). On the other hand, CBA/N mice survive *T. brucei* infection two or three times longer than conventional mice; the enhanced survival is not related to parasitemia, in accordance with the conclusions reached above, but is correlated with lower levels of circulating immune complexes (Gasbarre *et al.*, 1981b).

4.2. Observations in Cattle

These studies performed on genetically defined mice are not gentle pastimes for academic playboys, as certain have said, but are attempts to understand general mechanisms that have undubitable practical importance. Indeed, it appears that breeds of cattle in Africa also have varied levels of susceptibility to trypanosomiasis, and proposed introduction of less sensitive breeds in tsetse fly areas (reviewed in Finelle, 1973b) has raised a lot of interest and controversy.

Some biometricians hold as dogma or at least as a "well-known fact" that certain breeds of cattle, e.g., dwarf West African Shorthorn and N'Dama (described in details in Coulomb *et al.*, 1977) have a "racial aptitude to maintain themselves in good conditions and reproduce while harbouring trypanosomes without showing clinical sign of the disease" (Pagot, 1974; Pagot *et al.*, 1975; International Livestock Center for Africa, 1979; Choquel, 1969).

This phenomenon has been called "trypanotolerance"; however, there is ample evidence that this proposition is not quite correct: (1) it is not merely a racial trait, for Zebu and even Friesian (i.e., European) cattle can survive under high tsetse fly challenge under good conditions of husbandry (Stephen, 1966); (2) N'Dama cattle infected with trypanosomes are by no means in good conditions and do show clinical signs of the disease whether naturally or experimentally infected (Godfrey *et al.*, 1964; Stephen, 1966; Roberts and Gray, 1973b; Touré *et al.*, 1975; Murray *et al.*, 1977; Dargie *et al.*, 1979); (3) N'Dama cattle having recovered from infection naturally or after Berenil® treatment were found to be sterile from 4 to 32 months after apparent recovery (Ige and Amodu, 1975); (4) finally, it has been reported that under natural condition of exposure in tsetse fly areas, N'Dama cattle harbor very few trypanosomes (Chandler, 1952) which would make them actually trypano-"resistant." Thus, the term "trypanotolerance" is a misnomer and should be abandoned; in addition, it induces utter confusion in the mind of anybody who has been exposed to immunologic concepts, i.e., it has nothing to do with immune tolerance. For lack of a better word, and considering the tremendous range of variability in susceptibility in each breed under various conditions, it is suggested that one should speak about "greater" and "lesser" susceptibility of breeds of cattle.

This being said, the trend towards lesser susceptibility does definitely exist, and dwarf cattle are able to thrive under very poor conditions (Roberts and Gray, 1974), which may make them more resistant to disease in general. Further work on the nature and extent of lesser susceptibility, its biological basis, the possibility to use it practically and amplify it, as well as the possibility to transfer this character from dwarf cattle to the far more prestigious Zebu should be and are being pursued. This effort is even more justified if one considers that the lesser sensitivity "possessed by certain breeds of cattle against trypanosomiasis is generally and not merely against their own local strains," to use the words of

Stewart (1951), although it might be enhanced to homologous challenge by preinfection (Chandler, 1958).

Finally, considering individual rather than racial susceptibility on the one hand and the appeal Zebu cattle enjoy with farmers in Africa, the last words on this question could be left to Stephen (1966) who advocated more efforts in good husbandry for Zebus, and one should add selective breeding for lesser susceptibility rather than increased propagation of "slow-maturing, low milk- and meat-yielding dwarf breeds."

5. PROSPECTS

After almost a century of endeavors to eradicate African trypanosomiasis, one has clearly to realize that most efforts have been fruitless and that the prospects are bleak indeed. Medical teams are overburdened, faced with elusive patients, complex diagnosis, toxic drugs, and drug-resistant trypanosomes. Domestic animals in low tsetse fly challenge areas survive only under systematic drug treatment which is often unsatisfactory and impractical and is beginning to select drug-resistant parasites. Overall chemical pesticide campaigns have either failed at clearing tsetse flies or, in special cases, need constant and costly maintenance measures. New techniques such as the release of sterile males still need to be proven efficient in the practical field situation, and it is definitely necessary to reduce its cost. Finally, an immunologic solution, i.e., vaccination, is not in sight. Indeed, of all the "games parasites play" to evade immune surveillance (reviewed in Bloom, 1979), antigenic variation and immunodepression seem particularly efficient.

Pragmatic and empirical approaches are clearly not sufficient to cope with African trypanosomiasis. There is an absolute need, and indeed there is a move in that direction, for basic scientists to try to understand the mechanisms underlying the trypanosome life style and the diseases it causes. We believe that privileged areas of efforts should include (1) the elucidation of the extent and mechanism of antigenic variation, especially using DNA recombination technology to study variant antigen genomic organization, (2) the analysis of the extent and mechanism of immune depression, and (3) the genetic basis of lesser susceptibility of certain breeds of cattle to trypanosomiasis (Roelants, 1980). Moreover, as stated above, it is imperative that new drugs be developed to palliate immediate needs and that available drugs be used more rationally. Moves are being made by national and international organizations into these directions, but the scale of their efforts is, to date, not commensurate with the scale of the problem.

Finally, since the solution still seems to be far away, it might be wise to consider unemotionally how to avoid the possible catastrophic consequences on

the environment that could accompany trypanosomiasis eradication (Ormerod, 1976).

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REFERENCES

- Ackerman, S. B., and Seed, J. R., 1976a, Effect of *Trypanosoma brucei gambiense* infection in *Microtus montanus* on susceptibility to Ehrlich's tumours, *Infect. Immun.* 13:388–391.
- Ackerman, S. B., and Seed, J. R., 1976b, The effect of tryptophol on immune response and its implications toward trypanosome-induced immunosuppression, *Experientia* 32:645–647.
- Ackerman, S. B., and Seed, J. R., 1976c, Immuno-depression during *Trypanosoma brucei gambiense* infections in the field vole, *Microtus montanus*, *Clin. Exp. Immunol.* 25:152–158.
- Afchain, D., Le Ray, D., Van Miervenne, N., Fruit, J., and Capron, A., 1975, Analyse immunoelectrophorétique comparative des formes de culture et des formes sanguicoles de *Trypanosoma (Trypanozoon) brucei gambiense*. Caractérisation d'antigènes spécifiques de stade et de type antigénique, *Ann. Immunol.* 126C:45–50.
- Agabian, N., Thompson, L., Milhausen, M., and Stuart, K., 1980, Structural analysis of variant and invariant genes, *Am. J. Trop. Med. Hyg.* 29:1043–1052.
- Allsopp, B. A., and Njogu, A. R., 1974, Monosaccharide composition of the surface glycoprotein antigens of *Trypanosoma brucei*, *Parasitology* 69:271–281.
- Allsopp, B. A., Njogu, A. R., and Humphries, K. C., 1971, Nature and location of *Trypanosoma brucei* subgroup exoantigen and the relationship to 4S antigen, *Exp. Parasitol.* 29:271–284.
- Allt, G., Evans, E. M. E., Evans, D. H. L., and Targett, G. A. T., 1971, Effect of infection with trypanosomes on the development of experimental allergic neuritis in rabbits, *Nature* 233:197–199.
- Alwine, J. C., Kemp, D. J., and Stark, G. R., 1977, Methods for detection of specific RNAs in agarose gels by transfer to diazobenylmethoxypaper and hybridization with DNA probes, *Proc. Natl. Acad. Sci. U.S.A.* 74:5350–5354.
- Amole B. O., and Clarkson, A. B., 1981, *Trypanosoma brucei*: Host parasite interaction in parasite destruction by salicylhydroxamic acid and glycerol in mice, *Exp. Parasitol.*, submitted for publication.
- Amole, B. O., Clarkson, A. B., and Shear, H. L., 1981, Pathogenesis of anemia in *Trypanosoma brucei*-infected mice, submitted for publication.
- Anosa, V. O., and Isoun, T. T., 1976, Serum proteins, blood and plasma volumes in experimental *Trypanosoma vivax* infections of sheep and goats, *Trop. Anim. Health Prod.* 8:14–19.
- Askonas, B. A., Corsini, A. C., Clayton, C. E., and Ogilvie, B. M., 1979, Functional depletion of T- and B-memory cells and other lymphoid cell subpopulations during trypanosomiasis, *Immunology* 36:313–321.
- Askonas, B. A., Grosskinsky, C. M., and Sacks, D. L., 1981, Macrophages as mediators of immunosuppression in African trypanosomiasis, in: *A Century since Metchnikoff; Phagocytosis Past and Future*, Academic Press, New York, in press.
- Assoku, R. K. G., and Tizard, I. R., 1978, Mitogenicity of autolysates of *Trypanosoma congolense*, *Experientia* 34:127–129.

- Assoku, R. K. G., Hazlett, C. A., and Tizard, I., 1979, Immunosuppression in experimental African trypanosomiasis. Polyclonal B-cell activation and mitogenicity of trypanosome-derived saturated fatty acids, *Int. Arch. Allergy Appl. Immunol.* 59:298–307.
- Barbet, A. F., and McGuire, T. C., 1978, Crossreacting determinants in variant-specific surface antigens of African trypanosomes, *Proc. Natl. Acad. Sci. U.S.A.* 75:1989–1993.
- Barry, D., 1977, Loss of variant antigen by *Trypanosoma brucei* transforming *in vitro* and in tsetse fly, *J. Protozool.* 24:5A.
- Barry, J. D., 1979, Capping of variable antigen on *Trypanosoma brucei* and its immunological and biological significance, *J. Cell Sci.* 37:287–302.
- Barry, J. D., Hajduk, S. L., Vickerman, K., and Le Ray, D., 1979, Detection of multiple variable antigen types in metacyclic populations of *Trypanosoma brucei*, *Trans. R. Soc. Trop. Med. Hyg.* 73:205–208.
- Bauer, B., Iwannek, K. H., Hamann, H. J., and Adamsky, G., 1980, Use of gamma-irradiated blood for feeding tsetse flies, in: *Isotope and Radiation Research on Animal Diseases and Their Vectors*, International Atomic Energy Agency, Vienna, pp. 267–278.
- Bauer, F., 1956, Untersuchungen über die Wirkung des Di-(4 amidinophenyl) triazen-(N-1,3)-diacétural (Berenil), *Arzneim. Forsch.* 6:674–677.
- Bienen, E. J., Hammadi, E., and Hill, G. C., 1980, Initiation of trypanosome transformation from bloodstream trypomastigotes to procyclic trypomastigotes, *J. Parasitol.* 66:680–682.
- Bideau, J., Gidel, R., and Moity, J., 1966, Note préliminaire sur l'étude immuno-électrophorétique du sérum de bovins trypanosomés, *Bull. Soc. Pathol. Exot.* 59:817–825.
- Bloom, B. R., 1979, Games parasites play: How parasites evade immune surveillance, *Nature* 279:21–26.
- Bluestein, H. G., 1977, The role of histocompatibility region antigens in lymphocyte activation, in: *B and T Cells in Immune Recognition* (F. Looor and G. E. Roelants, eds.), John Wiley & Sons, London, pp. 291–311.
- Borst, P., Frasc, A. C. C., Bernards, A., Van der Ploeg, L. H. T., Hoeijmakers, J. H. J., Annberg, A. C., and Cross, G. A. M., 1981, DNA rearrangements involving the genes for variant antigens in *Trypanosoma brucei*, *Cold Spring Harbor Symp. Quant. Biol.* 45:935–944.
- Bouisset, L., Breuillaud, J., Carré, G., Larrouy, and Michel, G., 1968, Etude de l'ADN des trypanosomes, *Bull. Soc. Pathol. Exot.* 61:36–43.
- Brett, C. T., and Voorheis, H. P., 1980, Glycoprotein-biosynthesis in *Trypanosoma brucei*: The glycosylation of glycoprotein located in and attached to the plasma-membrane, *Eur. J. Biochem.* 109:139–150.
- Bridgen, P. J., Cross, G. A. M., and Bridgen, J., 1976, N-Terminal amino acid sequences of variant specific surface antigens from *Trypanosoma brucei*, *Nature* 263:613–614.
- Brown, K. N., and Williamson, J., 1962, Antigen of *brucei* trypanosomes, *Nature* 194:1253–1255.
- Browning, C. H., and Calver, K. M., 1943, The effect of the stage of infection on the chemotherapeutic response of *T. congolense* and on the immunity following cure, *J. Pathol. Bacteriol.* 55:393–394.
- Browning, C. H., and Gulbrandsen, R., 1936, Immunity following cure of experimental *Trypanosoma brucei* infection by a chemotherapeutic agent, *J. Pathol. Bacteriol.* 43:479–486.
- Campbell, G. H., and Phillips, S. M., 1976, Adoptive transfer of variant-specific resistance to *Trypanosoma rhodesiense* with B lymphocytes and serum, *Infect. Immun.* 14:1144–1150.
- Campbell, G. H., Esser, K. M., and Weinbaum, F. J., 1977, *Trypanosoma rhodesiense* infection in B cell deficient mice, *Infect. Immun.* 18:434–438.
- Campbell, G. H., Esser, K. M., and Phillips, S. N., 1978, *Trypanosoma rhodesiense* infection in congenitally athymic (nude) mice, *Infect. Immunol.* 20:714–720.
- Capbern, A., Giroud, C., Baltz, T., and Mattern, P., 1977, *Trypanosoma equiperdum*: Etude des variations antigéniques au cours de la trypanosomose expérimentale du lapin, *Exp. Parasitol.* 42:6–13.

- Challier, A., and Laveissière, C., 1973, Un nouveau piège pour la capture des glossines (*Glossina*, *Diptera*, *Museidae*): Description et essais sur le terrain, *Cah. O.R.S.T.O.M. Ser. Entomol. Med. Parasitol.* 1:251–262.
- Chandler, R. L., 1952, Comparative tolerance of West African N'Dama cattle to trypanosomiasis, *Ann. Trop. Med. Parasitol.* 46:127–134.
- Chandler, R. L., 1958, Studies on the tolerance of N'Dama cattle to trypanosomiasis, *J. Comp. Pathol.* 68:253–260.
- Choquel, P. G., 1969, Intérêt et utilisation des bovins trypanotolérants, Thesis, Ecole Nationale Vétérinaire, Alfort, Paris.
- Clarkson, A. B., and Brohn, F. H., 1976, Trypanosomiasis: An approach to chemotherapy by the inhibition of carbohydrate metabolism, *Science* 194:204–206.
- Clarkson, M. J., 1976, Immunoglobulin M in trypanosomiasis, in: *Pathophysiology of Parasitic Infections* (E. J. L. Soulsby, ed.), Academic Press, New York, pp. 171–182.
- Clarkson, M. J., and Penhale, W. J., 1973, Serum protein changes in trypanosomiasis in cattle, *Trans. R. Soc. Trop. Med. Hyg.* 62:273.
- Clarkson, M. J., Penhale, W. J., Edwards, G., and Farrel, P. F., 1975a, Serum protein changes in cattle infected with *Trypanosoma vivax*, *Trans. R. Soc. Trop. Med. Hyg.* 69:2.
- Clarkson, M. J., Penhale, W. J., and McKenna, R. B., 1975b, Progressive serum protein changes in experimental infections of calves with *Trypanosoma vivax*, *J. Comp. Pathol.* 85:397–410.
- Clayton, C. E., Ogilvie, B. M., and Askonas, B. A., 1979a, *Trypanosoma brucei* infection in nude mice: B lymphocyte function is suppressed in the absence of T lymphocytes, *Parasite Immunol.* 1:39–48.
- Clayton, C. E., Sacks, D. L., Ogilvie, B. M., and Askonas, B. A., 1979b, Membrane fractions of trypanosomes mimic the immunosuppressive and mitogenic effects of living parasites on the host, *Parasite Immunol.* 1:241–249.
- Corsini, A. C., Clayton, C., Askonas, B. A., and Ogilvie, B. M., 1977, Suppressor cells and loss of B-cells potential in mice infected with *Trypanosoma brucei*, *Clin. Exp. Immunol.* 29:122–131.
- Coulomb, J., Gruvel, J., Morel, P. C., Perreau, P., Quéval, R., Tibayrenc, R., and Provost, A., 1977, *La Trypanotolérance. Synthèse Bibliographique des Connaissances Actuelles*, Institut d'Élevage et de Médecine Vétérinaire des Pays Tropicaux, Paris.
- Cross, G. A. M., 1975, Identification, purification and properties of clone-specific glycoprotein antigens constituting the surface coat of *Trypanosoma brucei*, *Parasitology* 71:393–417.
- Cross, G. A. M., 1978, Antigenic variation in trypanosomes, *Proc. R. Soc. Lond. [Biol.]* 202:55–72.
- Cross, G. A. M., 1979a, Crossreacting determinants in the C-terminal region of trypanosome variant surface antigens, *Nature* 227:310–312.
- Cross, G. A. M., 1979b, Immunochemical aspects of antigenic variation in trypanosomes, *J. Gen. Microbiol.* 113:1–12.
- Cuisance, D., Politzar, H., Clair, M., Sellin, E., and Taze, Y., 1978, Impact des lâchers de mâles stériles sur les niveaux de deux populations sauvages de *Glossina palpalis gambiensis* en Haute-Volta (sources de la Volta Noire), *Rev. Elev. Med. Vet. Pays Trop.* 31:315–328.
- Cuisance, D., Politzar, H., Clair, M., Sellin, E., Taze, Y., Bourdoiseau, G., and Février, J., 1980, La lutte contre *Glossina palpalis gambiensis* Vanderplank par lâchers de mâles irradiés en Haute-Volta. Etude des paramètres opérationnels, in: *Isotope and Radiation Research on Animal Diseases and Their Vectors*, International Atomic Energy Agency, Vienna, pp. 249–264.
- Cunningham, M. P., 1968, Trypanosomiasis in African wild animals, *East Afr. Agric. Forestry J.* 33:264–265.
- Dallas, A. B. C., 1980, An Immunological Model of *Trypanosoma (Trypanozoon) brucei brucei* in the Vervet Monkey (*Cercopithecus aethiops*), Doctoral Thesis, University of Nairobi.
- Dame, D. A., Williamson, D. L., Cobb, P. E., Gates, D. B., Warner, P. V., Mtuya, A. G., and Baumgartner, H., 1980, Integration of sterile insects and pesticides for the control of the tsetse

- fly *Glossina morsitans morsitans*, in: *Isotope and Radiation Research on Animal Diseases and Their Vectors*, International Atomic Energy Agency, Vienna, pp. 267–278.
- Dar, R. K., Paris, J., and Wilson, A. J., 1973, Serological studies on trypanosomiasis in East Africa. IV. Comparison of antigenic types of *Trypanosoma vivax* group organisms, *Ann. Trop. Med. Parasitol.* 67:319–329.
- Dargie, J. D., Murray, P. K., Murray, M., Grimshaw, W., and McIntyre, W. I. M., 1979, Bovine trypanosomiasis: The red cell kinetics of N'Dama and Zebu cattle infected with *Trypanosoma congolense*, *Parasitology* 78:271–286.
- De Gee, A. L. W., and Shah, S. M., 1979, *Trypanosoma vivax* and immunosuppression, *Ann. Soc. Belge Med. Trop.* 59:199–201.
- De Gee, A. L. W., Ige, K., and Leeftang, P., 1976, Studies on *Trypanosoma vivax*: Transmission of mouse infective *T. vivax* by tsetse flies, *Int. J. Parasitol.* 6:419–421.
- De Gee, A. L. W., Shah, S. D., and Doyle, J. J., 1979, *Trypanosoma vivax*: Sequence of antigenic variants in mice and goats, *Exp. Parasitol.* 48:352–358.
- De Raadt, P., 1975, Review of African human trypanosomiasis, in: *International Scientific Council for Trypanosomiasis Research and Control, Fourteenth Meeting, Dakar, Senegal*, OAU/STRC, Nairobi, Publication No. 109, pp. 59–71.
- Diggs, C., Flemmings, B., Dillon, J., Snodgrass, R., Campbell, G., and Esser, K., 1976, Immune serum-mediated cytotoxicity against *Trypanosoma rhodesiense*, *J. Immunol.* 116:1005–1009.
- Dodin, A., Fromentin, H., and Gleye, M., 1962, Mise en évidence d'un antigène vaccinant dans le plasma de souris expérimentalement infectées par diverses espèces de trypanosomes, *Bull. Soc. Pathol. Exot.* 55:291–299.
- Donelson, J. E., Young, J. R., Majiwa, P. A. O., Shapiro, S. Z., Pennicavage, N. A., Marcu, K. B., and Williams, R. O., 1981, Comparison of the genes for two variable surface glycoproteins of *Trypanosoma brucei*, *J. Mol. Biol.*, in press.
- Doyle, J. J., 1977, Antigenic variation in the salivarian trypanosomes, in: *Advances in Experimental Medicine and Biology*, Volume 93: *Immunity to Blood Parasites of Animals and Man* (L. H. Miller, J. A. Pino, and J. J. McKelvey, eds.), Plenum Press, New York, pp. 31–63.
- Doyle, J. J., Hirumi, H., Hirumi, K., Lupton, E. N., and Cross, G. A. M., 1980, Antigenic variation in clones of animal-infective *Trypanosoma brucei* derived and maintained *in vitro*, *Parasitology* 80:359–369.
- Eardley, D. D., and Jayawardena, A. N., 1977, Suppressor cells in mice infected with *Trypanosoma brucei*, *J. Immunol.* 119:1029–1033.
- Esuruoso, G. O., 1976, The demonstration *in vitro* of the mitogenic effects of trypanosomal antigen on the spleen cells of normal, athymic and cyclophosphamide-treated mice, *Clin. Exp. Immunol.* 23:314–317.
- Evans, D. A., and Holland, M. F., 1978, Effective treatment of *Trypanosoma vivax* infections with salicylhydroxamic acid (SHAM), *Trans. R. Soc. Trop. Med. Hyg.* 72:203–204.
- Faghihi Shirazi, M., Holman, M., Hudson, K. M., Klaus, G. G. B., and Terry, R. J., 1980, Complement (C3) levels and the effect of C3 depletion in infections of *Trypanosoma brucei* in mice, *Parasite Immunol.* 2:155–161.
- Fairlamb, A. H., Oppendoes, F. R., and Borst, P., 1977, New approach to screening drugs for activity against African trypanosomes, *Nature* 265:270–271.
- Finelle, P., 1973a, Chimiothérapie et chimioprévention de la trypanosomiase animale. Acquisitions récentes et situation actuelle, *Cah. Med. Vet.* 42:215–226.
- Finelle, P., 1973b, La trypanosomiase africaine II. Chimioprévention, élevage de bétail trypanotolérant, *Rev. Mond. Zootech.* 8:24–27.
- Finelle, P., 1974, La trypanosomiase animale africaine. IV. Problèmes économiques, *Rev. Mond. Zootech.* 10:15–18.

- Finelle, P., 1975, Report on recent advances in animal trypanosomiasis, in: *International Scientific Council for Trypanosomiasis Research and Control, Fourteenth Meeting, Dakar, Senegal*, OAU/STRC, Nairobi, Publication No. 109, pp. 75–84.
- Finelle, P., 1980, Programme for the control of African animal trypanosomiasis and related development, in: *Isotope and Radiation Research on Animal Diseases and Their Vectors*, International Atomic Energy Agency, Vienna, pp. 3–14.
- Freeman, J. C., Hudson, K. M., and Byner, C., 1974, Immunosuppression in trypanosomiasis: Attempts to characterize the defect in the response of infected mice to sheep red blood cells, *Trans. R. Soc. Trop. Med. Hyg.* 68:149.
- Fulton, J. D., and Lourie, E. M., 1946, The immunity of mice cured of trypanosome infections, *Ann. Trop. Med. Parasitol.* 40:1–9.
- Gasbarre, L. C., Hug, K., and Louis, J. A., 1980, Murine T lymphocyte specificity for African trypanosomes. I. Induction of a T lymphocyte-dependent proliferative response to *Trypanosoma brucei*, *Clin. Exp. Immunol.* 41:97–106.
- Gasbarre, L. C., Hug, K., and Louis, J., 1981a, Murine T lymphocyte specificity for African trypanosomes. II. Suppression of the lymphocyte proliferative response to *Trypanosoma brucei* by systemic trypanosome infection, *Clin. Exp. Immunol.* 45:165–172.
- Gasbarre, L. C., Finerty, J. F., and Louis, J. A., 1981b, Nonspecific immune responses in CBA/N mice infected with *Trypanosoma brucei*, *Parasite Immunol.* 3:273–282.
- Geigy, R., Kauffmann, M., Mayende, J. S. P., Mwambu, P. M., and Onyango, R. J., 1973a, Isolation of *Trypanosoma (Trypanozoon) rhodesiense* from game and domestic animals in Musoma District, Tanzania, *Acta Trop. (Basel)* 30:49–56.
- Geigy, R., Mwambu, P. M., and Kauffmann, M., 1973b, Sleeping sickness survey in Musoma District, Tanzania. IV. Examination of wild mammals as a potential reservoir for *T. rhodesiense*, *Acta Trop. (Basel)* 28:211–220.
- Gershon, R. K., Eardley, D. D., Green, D. R., Shen, F. W., Yamauchi, K., Cantor, H., and Murphy, D. B., 1981, Contrasuppression: A novel immunoregulatory activity, *J. Exp. Med.* 153:1533–1546.
- Giannini, M. S., and D'Alessandro, P. A., 1978, Unusual antibody-induced modulation of surface antigens in the cell coat of a bloodstream trypanosome, *Science* 201:916–918.
- Gibson, W. C., Marshall, T. F. de C., and Godfrey, D. C., 1980, Numerical analysis of enzyme polymorphism: A new approach to the epidemiology and taxonomy of trypanosomes of the subgenus *Trypanosoma*, *Adv. Parasitol.* 18:175–246.
- Gidel, R., 1962, Etude électrophorétique quantitative en gélose des protéines sériques des bovins, *Rev. Elev. Med. Vet. Pays Trop.* 15:259–263.
- Godfrey, D. G., Leach, T. M., and Killick-Kendrick, R., 1964, Bovine trypanosomiasis in Nigeria. III. A high incidence in a group of West African humpless cattle, *Ann. Trop. Med. Parasitol.* 58:204–215.
- Goodwin, L. G., 1970, The pathology of African trypanosomiasis, *Trans. R. Soc. Trop. Med. Hyg.* 64:797–812.
- Goodwin, L. G., Green, D. G., Guy, M. W., and Voller, A., 1972, Immunosuppression during trypanosomiasis, *Br. J. Exp. Pathol.* 53:40–43.
- Gray, A. R., 1962, The influence of antibody on serological variation in *Trypanosoma brucei*, *Ann. Trop. Med. Parasitol.* 56:4–13.
- Gray, A. R., 1965, Antigenic variation in a strain of *Trypanosoma brucei* transmitted by *Glossina morsitans* and *G. palpalis*, *J. Gen. Microbiol.* 41:195–214.
- Gray, A. R., 1966, The antigenic relationship of strains of *Trypanosoma brucei* isolated in Nigeria, *J. Gen. Microbiol.* 44:263–271.
- Gray, A. R., 1970, A study of the antigenic relationships of isolates of *Trypanosoma brucei* collected from a herd of cattle kept in one locality for five years, *J. Gen. Microbiol.* 62:301–313.

- Gray, A. R., 1975, A pattern in the development of agglutinogenic antigens of cyclically transmitted isolates of *Trypanosoma gambiense*, *Trans. R. Soc. Trop. Med. Hyg.* 69:131–138.
- Greenwood, B. M., 1974a, Possible role of a B-cell mitogen in hypergammaglobulinaemia in malaria and trypanosomiasis, *Lancet* 1:435–436.
- Greenwood, B. M., 1974b, Immunosuppression in patients with malaria and sleeping sickness, *Ciba Found. Symp.* 25:138–146.
- Greenwood, B. M., Whittle, H. C., and Molyneux, D. H., 1973, Immunosuppression in Gambian trypanosomiasis, *Trans. R. Soc. Med. Hyg.* 67:846–850.
- Griffin, L., 1978, African trypanosomiasis in sheep and goats: A review, *Vet. Bull.* 48:819–825.
- Herbert, W. J., 1975, Interference between two strains of *Trypanosoma brucei*, *Trans. R. Soc. Trop. Med. Hyg.* 69:272.
- Herbert, W. J., Parratt, D., Van Meirvenne, N., and Lennox, B., 1980, An accidental laboratory infection with trypanosomes of a defined stock. II. Studies on the serological response of the patient and the identity of the infecting organism, *J. Infect.* 2:113–124.
- Hicks, J. B., Stathern, J., and Hershowitz, I., 1977, The cassette model of making type interconversions, *Cold Spring Harbor Symp. Quant. Biol.* 39:457–468.
- Hill, G. C., 1980, Biochemical studies using *Trypanosoma rhodesiense* culture-infective trypanomastigotes, in: *The Host Invader Interplay* (H. Van den Bossche, ed.), Janssen Research Foundation/Elsevier/North-Holland Biomedical Press, Amsterdam, pp. 555–566.
- Hill, G. C., Shimer, S., Caughey, B., and Saver, S., 1977, Growth of infective forms of *Trypanosoma rhodesiense* in vitro, the causative agent of African trypanosomiasis, *Science* 196:992–994.
- Hirumi, H., Doyle, J. J., and Hirumi, K., 1977a, Cultivation of bloodstream *Trypanosoma brucei*, *Bull. WHO* 55:405–409.
- Hirumi, H., Doyle, J. J., and Hirumi, K., 1977b, African trypanosomes: Cultivation of animal-infective *Trypanosoma brucei* in vitro, *Science* 196:992–994.
- Hirumi, H., Hirumi, K., Doyle, J. J., and Cross, G. A. M., 1980, In vitro cloning of animal-infective bloodstream forms of *Trypanosoma brucei*, *Parasitology* 80:371–382.
- Hoare, C. A., 1972, *The Trypanosomes of Mammals*, Blackwell Scientific, Edinburgh, Oxford.
- Hoeijmakers, J. H. J., Frasch, A. C. C., Bernards, A., Borst, P., and Cross, G. A. M., 1980, Novel expression-linked copies of the genes for variant surface antigens in trypanosomes, *Nature* 284:78–80.
- Holmes, P. H., Mammo, C., Thomson, A., Knight, P. A., Lucken, R., Murray, P. K., Murray, M., Jennings, F. W., and Urquhart, G. M., 1974, Immunosuppression in bovine trypanosomiasis, *Vet. Rec.* 95:86–87.
- Hornby, H. E., 1921, Trypanosomes and trypanosomiasis of cattle, *J. Comp. Pathol.* 34:211–240.
- Hornby, H. E., 1941, Immunisation against bovine trypanosomiasis, *Trans. R. Soc. Trop. Med. Hyg.* 35:165–176.
- Houba, V., Brown, K. N., and Allison, A. C., 1969, Heterophile antibodies, M-anti-globulins and immunoglobulins in experimental trypanosomiasis, *Clin. Exp. Immunol.* 4:113–123.
- Hu, C. H., 1931, The lymphatic reaction in experimental trypanosomiasis, *Natl. Med. J. China* 17:435–457.
- Hudson, K. M., and Terry, R. J., 1979, Immunodepression and the course of infection of a chronic *Trypanosoma brucei* infection in mice, *Parasite Immunol.* 1:317–326.
- Hudson, K. M., Byner, C., Freeman, J., and Terry, R. J., 1976, Immunodepression, high IgM levels and evasion of the immune response in murine trypanosomiasis, *Nature* 264:256–258.
- Hudson, K. M., Taylor, A. E. R., and Elce, B. J., 1980, Antigenic changes in *Trypanosoma brucei* on transmission by tsetse fly, *Parasite Immunol.* 2:57–69.
- Ige, K., and Amodu, A. A., 1975, Studies on the pathogenesis of trypanosomiasis for N'Dama cattle, in: *International Scientific Council for Trypanosomiasis Research and Control Fourteenth Meeting, Dakar, Senegal*, OAU/STRC, Nairobi, Publication No. 109, pp. 151–156.

- International Atomic Energy Agency, 1980, *Isotope and Radiation Research on Animal Diseases and Their Vectors, Proceedings of a Symposium*, IAEA, Vienna.
- International Livestock Center for Africa, 1979, *Trypanotolerant Livestock in West and Central Africa*, Volume I: General Study, International Livestock Center for Africa, Addis-Ababa.
- Itard, J., and Clair, M., 1975, Project to control tsetse flies by the release of sterile males at Bobo-Dioulasso (Upper-Volta), in: *International Scientific Council for Trypanosomiasis Research and Control, Fourteenth Meeting, Dakar, Senegal*, Nairobi, OAU/STRC Publication No. 109, pp. 375–378.
- Jayawardena, A. N., and Waksman, B. H., 1977, Suppressor cells in experimental trypanosomiasis, *Nature* 265:539–541.
- Jayawardena, A. N., Waksman, B. H., and Eardley, D. D., 1978, Activation of distinct helper and suppressor T-cells in experimental trypanosomiasis, *J. Immunol.* 121:622–628.
- Jenni, L., 1977a, Comparisons of antigenic types of *Trypanosoma (T.) brucei* strains transmitted by *Glossina m. morsitans*, *Acta Trop. (Basel)* 34:35–41.
- Jenni, L., 1977b, Antigenic variants in cyclically transmitted strains of the *T. brucei* complex, *Ann. Soc. Belge Med. Trop.* 57:383–386.
- Jennings, F. W., Whitelaw, D. D., Holmes, P. H., and Urquhart, G. M., 1978, The susceptibility of strains of mice to infection with *Trypanosoma congolense*, *Res. Vet. Sci.* 25:399–400.
- Jones, T. W., and Clarkson, M. J., 1972a, The effect of tsetse passage on variants of *Trypanosoma vivax*, *Trans. R. Soc. Trop. Med. Hyg.* 66:336.
- Jones, T. W., and Clarkson, M. J., 1972b, The effect of syringe and cyclical passage on antigenic variants of *Trypanosoma vivax*, *Ann. Trop. Med. Parasitol.* 66:303–312.
- Jones, T. W., and Clarkson, M. J., 1974, The timing of antigenic variation in *Trypanosoma vivax*, *Ann. Trop. Med. Parasitol.* 68:485–486.
- Kaaya, G. P., Valli, V. E. O., Maxie, M. G., and Losos, G. J., 1979, Inhibition of bovine bone marrow granulocyte/macrophage colony formation *in vitro* by serum collected from cattle infected with *Trypanosoma vivax* or *Trypanosoma congolense*, *Tropenmed. Parasitol.* 30:230–235.
- Kanthack, A. A., Durham, H. E., and Blandford, W. F. H., 1898, On nagana, or tsetse fly disease, *Proc. R. Soc.* 64:100–118.
- Kar, S. K., Roelants, G. E., Mayor-Withey, K. S., and Pearson, T. W., 1981, Immune depression in trypanosome-infected mice. VI. Comparison of immune responses of different lymphoid organs, *Eur. J. Immunol.* 11:100–105.
- Kar, S. K., Murray, M., Roelants, G. E., Logan, K. S., and Morrison, W. I., 1981b, Immune depression in trypanosome-infected mice. VII. Comparison of the immune suppression generated in spleen and lymph nodes by *T. brucei* and *T. congolense* in two strains of mice, submitted for publication.
- Kempt, J., Louis, D., Mattingly, J., Bennet, J., Higuchi, C., Horowitz, M., and Gershon, R. K., 1981, Suppressor cells *in vitro*: Differential effects of indomethacin and related compounds, *J. Immunopharmacol.* 2:471–489.
- Kosinski, R. J., 1980, Antigenic variation in trypanosomes: A computer analysis of variant order, *Parasitology* 80:343–357.
- Laird, M., 1975, Recent developments in vector control as applied to the tsetse problem, in: *International Scientific Council for Trypanosomiasis Research and Control, Fourteenth Meeting, Dakar, Senegal*, Nairobi, OAU/STRC Publication No. 109, pp. 331–334.
- Laveran, A., 1911, Résistance des chèvres et des moutons aux trypanosomiasis: Longue durée de l'immunité acquise à la suite de ces maladies, *C. R. Acad. Sci. (Paris)* 145:63–66.
- Leefflang, P., Buys, J., and Blotkamp, J., 1976, Studies on *Trypanosoma vivax* infectivity and serial maintenance of natural bovine isolates in mice, *Int. J. Parasitol.* 6:413–417.
- Le Ray, D., Barry, J. D., Easton, C., and Vickerman, K., 1977, First tsetse fly transmission of the "Antat" serodeme of *Trypanosoma brucei*, *Ann. Soc. Belge Med. Trop.* 57:369–381.

- Le Ray, D., Barry, J. D., and Vickerman, K., 1978, Antigenic heterogeneity of metacyclic forms of *Trypanosoma brucei*, *Nature* 273:300–302.
- Levaditi, C., and McIntosh, J., 1910, Le Mécanisme de la création des variétés de trypanosomes résistants aux anticorps, *C. R. Soc. Biol. (Paris)* 66:49–51.
- Lilly, F., and Pincus, T., 1973, Genetic control of murine viral leukaemogenesis, *Adv. Cancer Res.* 17:231–277.
- Longstaffe, J. A., Freeman, J., and Hudson, K. M., 1973, Immunosuppression in trypanosomiasis: Some thymus dependent and thymus independent responses, *Trans. R. Soc. Trop. Med. Hyg.* 67:264.
- Loor, F., 1980, Plasma membrane and cell cortex interactions in lymphocyte functions, *Adv. Immunol.* 30:1–120.
- Loor, F., and Roelants, G. E., 1974, High frequency of lymphocytes of T lineage in nude mouse spleen, *Nature* 251:229–230.
- Loor, F., and Roelants, G. E., 1975, Immunofluorescence studies of a possible pre-thymic T-cell differentiation in congenitally athymic (nude) mice, *Ann. N.Y. Acad. Sci.* 54:226–241.
- Loor, F., Hägg, L.-B., Mayor, K. S., and Roelants, G. E., 1975, θ -Positive cells in nude mice born from a homologous nu/nu mother, *Nature* 255:657–658.
- Loor, F., Amstutz, H., Hägg, L.-B., Mayor-Withey, K. S., and Roelants, G. E., 1976, T lineage lymphocytes in nude mice born from homozygous nu/nu parents, *Eur. J. Immunol.* 6:663–665.
- Luckins, A. G., 1972a, Adaptive immunity in experimental trypanosomiasis, *Trans. R. Soc. Trop. Med. Hyg.* 66:346–347.
- Luckins, A. G., 1972b, Studies on bovine trypanosomiasis, serum immunoglobulin levels in Zebu cattle exposed to natural infection in East Africa, *Br. Vet. J.* 128:523–528.
- Luckins, A. G., 1976, The immune response of Zebu cattle to infection with *Trypanosoma congolense* and *T. vivax*, *Ann. Trop. Med. Parasitol.* 70:133–145.
- Luckins, A. G., 1977, Detection of antibodies in trypanosome infected cattle by means of a micropate linked immunosorbent assay, *Trop. Anim. Health Prod.* 9:53–62.
- Luckins, A. G., and Mehlitz, D., 1976, Observations on serum immunoglobulin levels in cattle infected with *Trypanosoma brucei*, *T. vivax* and *T. congolense*, *Ann. Trop. Med. Parasitol.* 70:479–480.
- Lumsden, W. H. R., Herbert, W. J., and Hardy, G. J. C., 1965, *In vivo* prophylactic activity of Berenil against trypanosomes in mice, *Vet. Rec.* 5:147–148.
- Lumsden, W. H. R., Herbert, W. J., and McNeillage, G. J. C., 1967, Nomenclature of antigenic types of trypanosomes, *Vet. Rec.* 81:237–238.
- Lumsden, W. H. R., Herbert, W. J., and McNeillage, G. J. C., 1973, *Techniques with Trypanosomes*, Churchill Livingstone, Edinburgh, London.
- Macaskil, J. A., Holmes, P. H., Whitelaw, D. D., McConnel, I., Jennings, F. W., and Urquhart, G. M., 1980, Immunological clearance of ^{75}Se -labeled *Trypanosoma-brucei* in mice. 2. Mechanisms in immune animals, *Immunology* 40:629–635.
- MacLennan, K. J. R., and Na'Isa, B. K., 1970, Relapsing *T. vivax* infections in Nigerian Zebu cattle treated with diminazene aceturate, *Trop. Anim. Health Prod.* 2:189–195.
- Mackenzie, A. R., Sibley, P. R., and White, B. P., 1979, Differential suppression of experimental allergic disease in rats infected with trypanosomes, *Parasite Immunol.* 1:49–59.
- Mackenzie, P. K. I., Boyt, W. P., Emslie, V. W., Lander, K. P., and Swanepoel, R., 1975, Immunosuppression in ovine trypanosomiasis, *Vet. Rec.* 97:452–453.
- Mackenzie, P. K. I., Boyt, W. P., and Nesham, V. W., 1979, Serum immunoglobulin levels in sheep during the course of naturally acquired and experimentally induced trypanosomiasis, *Br. Vet. J.* 135:178–184.
- Magnus, E., Vervoort, T., and Van Meirvenne, V., 1978, A card-agglutination test with stained

- trypanosomes (C.A.T.T.) for the serological diagnosis of *T. b. gambiense* trypanosomeiasis, *Ann. Soc. Belge Med. Trop.* 58:169–176.
- Mansfield, J. M., and Bagasra, O., 1978, Lymphocyte function in experimental African trypanosomiasis. I. B cell responses to helper T-cell-independent and -dependent antigens, *J. Immunol.* 120:759–765.
- Mansfield, J. M., and Kreier, J. P., 1972a, Autoimmunity in experimental *Trypanosoma congolense* infections of rabbits, *Infect. Immun.* 5:648–656.
- Mansfield, J. M., and Kreier, J. P., 1972b, Tests for antibody- and cell-mediated hypersensitivity to trypanosome antigens in rabbit infected with *Trypanosoma congolense*, *Infect. Immun.* 6:62–67.
- Mansfield, J. M., and Wallace, J. H., 1974, Suppression of cell-mediated immunity in experimental African trypanosomiasis, *Infect. Immun.* 10:335–339.
- Mansfield, J. M., Craig, S. A., and Stelzer, G. T., 1976, Lymphocyte function in experimental African trypanosomiasis: Mitogenic effects of trypanosome extracts *in vitro*, *Infect. Immun.* 14:976–981.
- Marcu, K. B., and Williams, R. O., 1981, Microbial surface elements: The case of variant surface glycoproteins (VSG) genes of African trypanosomes, in: *Genetic Engineering*, Volume 3 (J. K. Setlow and A. Hollaender, eds.), Plenum Press, New York, pp. 129–156.
- Margulis, L., 1976, Genetic and evolutionary consequences of symbiosis, *Exp. Parasitol.* 39:277–349.
- Masake, R. A., and Morrison, W. I., 1981, An evaluation of the structural and functional changes in the lymphoid organs of Boran cattle infected with *Trypanosoma vivax*, *Am. J. Vet. Res.* 42:1738–1746.
- Masake, R. A., Pearson, T. W., Wells, P., and Roelants, G. E., 1981, The *in vitro* response to mitogens of leukocytes from cattle infected with *Trypanosoma congolense*, *Clin. Exp. Immunol.*, 43:583–589.
- Mattern, P., Masseyeff, R., Michel, R., and Peretti, P., 1978, Etude immunochimique de la 2-macroglobuline des sérums de malades atteints de trypanosomiase africaine à *T. gambiense*, *Ann. Immunol. (Paris)* 101:382–393.
- Mayor-Withey, K. S., Clayton, C. E., Roelants, G. E., and Askonas, B. A., 1978, Trypanosomiasis leads to extensive proliferations of B, T and null cells in spleen and bone marrow, *Clin. Exp. Immunol.* 34:359–363.
- McDevitt, H. O., Oldstone, M. B. A., and Pincus, T., 1974, Histocompatibility-linked genetic control of specific immune responses to viral infections, *Transplant. Rev.* 19:209–225.
- Merritt, S. C., 1980, Purification and cell-free translation of mRNA coding for a variant specific antigen from *Trypanosoma brucei gambiense*, *Mol. Biochem. Parasitol.* 1:151–166.
- Mesnil, E., and Brimont, E., 1909, Propriétés protectrices du sérum des animaux trypanosomés: Races résistantes à ces sérums, *Ann. Inst. Pasteur* 23:129–154.
- Mitchell, G. F., 1978, Metazoon and protozoon parasitic infections in nude mice, *Contemp. Top. Immunobiol.* 8:55–67.
- Mitry-Martin, L., Hudson, K. M., and Terry, R. J., 1979, Autoantibodies to splenocytes in murine trypanosomiasis, *Trans. R. Soc. Trop. Med. Hyg.* 73:98.
- Molyneux, D. H., 1976, Vector relationship in the *Trypanosomatidae*, *Adv. Parasitol.* 15:1–82.
- Molyneux, D. H., Baldry, D. A. T., and Fairhurst, C., 1979, Tsetse movements in wind fields. Possible epidemiological and entomological implications for trypanosomiasis and its control, *Acta Trop. (Basel)* 36:53–65.
- Morrison, W. I., and Murray, M., 1979a, Lymphoid changes in African trypanosomiasis, in: *Pathogenicity of Trypanosomes* (G. Losos and A. Chouinard, eds.), International Development Research Center, Ottawa, pp. 154–160.

- Morrison, W. I., and Murray, M., 1979b, *Trypanosoma congolense*: Inheritance of the susceptibility to infection in inbred strains of mice, *Exp. Parasitol.* 48:364–374.
- Morrison, W. I., Roelants, G. E., Mayor-Withey, K. S., and Murray, M., 1978, Susceptibility of inbred strains of mice to *Trypanosoma congolense*: Correlation with changes in spleen lymphocyte populations, *Clin. Exp. Immunol.* 32:25–40.
- Morrison, W. I., Roelants, G. E., Pearson, T. W., and Murray, M., 1979, Genetic control of susceptibility to *T. congolense* infection in inbred strain of mice, in: *Function and Structure of the Immune System* (W. Müller-Ruchholtz and H. K. Müller-Hermelink, eds.), Plenum Press, New York, pp. 709–714.
- Moulton, J. E., and Coleman, J. L., 1979, A soluble immunosuppressor substance in spleen in deer mice infected with *Trypanosoma brucei*, *Am. J. Vet. Res.* 40:1131–1133.
- Mulligan, H. W., 1970, *The African Trypanosomiasis*, Allen and Unwin, London.
- Murray, M., 1974, The pathology of African trypanosomiasis, in: *Progress in Immunology* (L. Brent and J. Holborow, eds.), North Holland, Amsterdam, pp. 181–192.
- Murray, M., 1979, Anemia of bovine African trypanosomiasis: An overview, in: *Pathogenicity of Trypanosomes* (G. Losos and A. Chouinard, eds.), International Development Research Center, Ottawa, pp. 71–81.
- Murray, M., and Morrison, W. I., 1979a, Non-specific induction of increased resistance in mice to *Trypanosoma congolense* and *Trypanosoma brucei* by immunostimulants, *Parasitology* 79:349–366.
- Murray, M., and Morrison, W. I., 1979b, Parasitemia and host susceptibility to African trypanosomiasis, in: *Pathogenicity of Trypanosomes* (G. Losos and A. Chouinard, eds.), International Development Research Center, Ottawa, pp. 71–81.
- Murray, P. K., Urquhart, G. M., Murray, M., and Jennings, F. W., 1973, The response of mice infected with *T. brucei* to the administration of sheep erythrocytes, *Trans. R. Soc. Trop. Med. Hyg.* 67:267.
- Murray, P. K., Jennings, F. W., Murray, M., and Urquhart, G. M., 1974a, The nature of immunosuppression in *Trypanosoma brucei* infections in mice. I. The role of the macrophage, *Immunology* 27:815–824.
- Murray, P. K., Jennings, F. W., Murray, M., and Urquhart, G. M., 1974b, The nature of immunosuppression in *Trypanosoma brucei* infections in mice. II. The role of the T and B lymphocytes, *Immunology* 27:825–840.
- Murray, P. K., Murray, M., Wallace, M., Morrison, W. I., and McIntire, W. I. M. 1977, An experimental investigation of susceptibility to *Trypanosoma brucei*, *T. congolense* and mixed infections, in: *International Scientific Council for Trypanosomiasis Research and Control, Proceedings of the 15th Meeting, The Gambia*, OAU/STRC, Nairobi, pp. 470–481.
- Murray, M., Morrison, W. I., Emery, D. L., Akol, G. W. O., Masake, R. A., and Moloo, S. K., 1980, Pathogenesis of trypanosome infections in cattle, in: *Isotope and Radiation Research on Animal Diseases and Their Vectors*, International Atomic Energy Agency, Vienna, pp. 15–30.
- Musoke, A. J., and Barbet, A. F., 1977, Activation of complement by variant-specific surface antigen of *Trypanosoma brucei*, *Nature* 270:438–440.
- Mwambu, P. M., Wafula, R. Z. O., Mayende, J. S. P., and Kavemba, L., 1975, A preliminary report on the incidence of trypanosomiasis in domestic and game animals in Kigouna region of Tanzania and the role these animals play as reservoir hosts of the human parasite, in: *International Scientific Council for Trypanosomiasis Research and Control, Fourteenth Meeting, Dakar, Senegal*, OAU/STRC, Nairobi, Publication No. 109, pp. 163–166.
- Nantulya, V. M., and Doyle, J. J., 1977, Stabilisation and preservation of the antigenic specificity of *Trypanosoma (Trypanozoon) brucei* variant specific surface antigens by mild fixation techniques, *Acta Trop. (Basel)* 34:313–320.

- Nantulya, V. M., Musoke, A. J., Barbet, A. F., and Roelants, G. E., 1979, Evidence for reappearance of *Trypanosoma brucei* variable antigen types in relapse populations, *J. Parasitol.* 65:673–679.
- Nantulya, V. M., Doyle, J. J., and Jenni, L., 1980a, Studies on *Trypanosoma (Nannomonas) congolense*. III. Antigenic variation in three cyclically transmitted stocks, *Parasitology* 80:123–131.
- Nantulya, V. M., Doyle, J. J., and Jenni, L., 1980b, Studies on *Trypanosoma (Nannomonas) congolense*. IV. Experimental immunization of mice against tsetse fly challenge, *Parasitology* 80:133–137.
- Ogilvie, B. M., and Wilson, R. J. M., 1976, Evasion of the immune response by parasites, *Br. Med. Bull.* 32:177–181.
- Ormerod, W. E., 1976, Ecological effect of control of African trypanosomiasis, *Science* 191:815–821.
- Pagot, J., 1974, Les races trypanotolérantes, *Rev. Elev. Med. Vet. Pays Trop.* 27:235–248.
- Pagot, J., Coulomb, J., and Petit, J. P., 1975, Review and present situation of the use of trypanotolerant breeds, in: *International Scientific Council for Trypanosomiasis Research and Control, Fourteenth Meeting, Dakar, Senegal*, OAU/STRC, Nairobi, Publication No. 109, pp. 119–140.
- Pearson, T. W., Roelants, G. E., Lundin, L. B., and Mayor-Withey, K. S., 1978, Immune depression in trypanosome-infected mice. I. Depressed T lymphocyte responses, *Eur. J. Immunol.* 8:723–727.
- Pearson, T. W., Roelants, G. E., Pinder, M., Lundin, L. B., and Mayor-Withey, K. S., 1979, Immune depression in trypanosome-infected mice. III. Suppressor cells, *Eur. J. Immunol.* 9:200–204.
- Pearson, T. W., Pinder, M., Roelants, G. E., Kar, J. K., Lundin, L. B., Mayor-Withey, K. S., and Hewett, R. S., 1980, Methods for derivation and detection of anti-parasite monoclonal antibodies, *J. Immunol. Meth.* 34:141–154.
- Pearson, T. W., Kar, S. K., McGuire, T. C., and Lundin, L. B., 1981, Trypanosome variable surface antigens: Studies using two-dimensional gel electrophoresis and monoclonal antibodies, *J. Immunol.* 126:823–928.
- Pearson, T. W., Morrison, W. I., Roelants, G. E., Lundin, L. B., and Mayor-Withey, K. S., 1982, Immune depression in trypanosome-infected mice. V. Correlation of immune suppression with susceptibility to disease in various inbred strains of mice, submitted.
- Politzar, H., Cuisance, D., Clair, M., Taze, Y., Sellin, E., and Bourdoiseau, G., 1980, Etude préliminaire sur la longévité et la dispersion des mâles irradiés de *Glossina palpalis gambiensis* dans leur biotope naturel (Haute-Volta), *Rev. Elev. Med. Vet. Pays Trop.* 33:45–50.
- Reid, H. W., Holmes, P. H., and Skinner, H. H., 1979, Immunosuppression in experimental trypanosomiasis: Effects of *Trypanosoma brucei* on immunization against louping-ill virus and lymphocytic choriomeningitis virus, *J. Comp. Pathol.* 89:581–585.
- Roberts, C. J., and Gray, A. R., 1973a, Studies on trypanosome resistant cattle. I. The breeding and growth performance of N'Dama, Muturu and Zebu cattle maintained under the same conditions of husbandry, *Trop. Anim. Health Prod.* 5:211–219.
- Roberts, C. J., and Gray, A. R., 1973b, Studies on trypanosome resistant cattle. II. The effect of trypanosomiasis on N'Dama, Muturu and Zebu cattle, *Trop. Anim. Health Prod.* 5:220–233.
- Roberts, C. J., and Gray, A. R., 1974, The resistance of N'Dama, Muturu and Zebu cattle to trypanosomiasis, in: *Report of the International Scientific Council for Trypanosomiasis Research, Thirteenth Meeting, Lagos*, OAU/STRC, Nairobi, Publication No. 105, pp. 97–101.
- Roelants, G. E., 1980, Prospects in the immunology of trypanosomiasis, in: *Report of the Expert Consultation on Research on Trypanosomiasis*, Food and Agriculture Organization of the United Nations, Rome, pp. 75–79.

- Roelants, G. E., and Mayor-Withey, K. S., 1977, The regulation of T lymphocyte precursor pool by a thymic humoral factor, *Cell. Immunol.* 34:420-423.
- Roelants, G. E., Loor, F., von Boehmer, H., Sprent, J., Hägg, L.-B., Mayor, K. S., and Ryden, A., 1975, Five types of lymphocytes ($Ig^{+}\theta^{-}$, $Ig^{-}\theta^{+}$ weak, $Ig^{-}\theta^{+}$ strong, $Ig^{-}\theta^{-}$ and $Ig^{+}\theta^{+}$) characterized by double immunofluorescence and electrophoretic mobility. Organ distribution in normal and nude mice, *Eur. J. Immunol.* 5:127-131.
- Roelants, G. E., Mayor, K. S., Hägg, L.-B., and Loor, F., 1976, Immature T lineage lymphocytes in athymic mice. Presence of TL, lifespan and homeostatic regulation, *Eur. J. Immunol.* 6:75-81.
- Roelants, G. E., Pearson, T. W., Morrison, W. I., Mayor-Withey, K. S., and Lundin, L. B., 1979a, Immune depression in trypanosome infected mice. IV. Kinetics of suppression and alleviation by the trypanocidal drug Berenil, *Clin. Exp. Immunol.* 37:457-469.
- Roelants, G. E., Pearson, T. W., Tyrer, H. W., Mayor-Withey, K. S., and Lundin, L. B., 1979b, Immune depression in trypanosome-infected mice. II. Characterization of the spleen cells involved, *Eur. J. Immunol.* 9:195-199.
- Rovis, L., and Baekkeskov, S., 1980, Subcellular fractionation of *Trypanosoma brucei*. Isolation and characterization of plasma-membranes, *Parasitology* 80:507-524.
- Rovis, L., and Dube, D. K., 1981a, Studies on the biosynthesis of the variant surface glycoprotein of *Trypanosoma brucei*: Sequence of glycosylation, *Mol. Biochem. Parasitol.* 4:77-93.
- Rovis, L., and Dube, S., 1981b, I. Identification and characterization of two N-acetylglucosaminyl transferases associated with *T. Brucei* microsomes, *Mol. Biochem. Parasitol.*, in press.
- Rovis, L., Barbet, A. F., and Williams, R. O., 1978, Characterization of the surface coat of *Trypanosoma congolense*, *Nature* 271:654-656.
- Rurangirwa, F. R., Tabel, H., Losos, G., Nasiga, W. N., and Mwambu, P., 1978, Immunosuppressive effect of *Trypanosoma congolense* and *Trypanosoma vivax* on the secondary immune response of cattle to *Mycoplasma mycoides* subsp. *mycoides*, *Res. Vet. Sci.* 25:395-397.
- Rurangirwa, F. R., Tabel, H., Losos, G. J., and Tizard, I. R., 1979, Suppression of antibody response to *Leptospira biflexa* and *Brucella abortus* and recovery from immunosuppression after Berenil treatment, *Infect. Immun.* 26:822-826.
- Rurangirwa, F. R., Mushi, E. Z., Tabel, H., Tizard, J. R., and Losos, G. J., 1980a, The effect of *Trypanosoma congolense* and *T. vivax* infections on the antibody response of cattle to live rinderpest virus vaccine, *Res. Vet. Sci.* 28:264-266.
- Rurangirwa, F. R., Tabel, H., Losos, G. J., and Tizard, I. R., 1980b, Immunosuppression in bovine trypanosomiasis. The establishment of "memory" in cattle infected with *T. congolense* and the effect of post infection serum on *in vitro* ^3H -thymidine uptake by lymphocytes and on leukocyte migration, *Tropenmed. Parasitol.* 31:105-110.
- Rurangirwa, F. R., Tabel, H., Losos, G., and Tizard, I. R., 1980c, Haemolytic complement and serum C3 levels in Zebu cattle infected with *Trypanosoma congolense* and *Trypanosoma vivax* and the effect of trypanocidal treatment, *Infect. Immun.* 27:832-836.
- Ryan, L., and Molyneux, D. H., 1980, Construction details of the Challier/Laveissière biconical trap, in: *Isotope and Radiation Research on Animal Diseases and Their Vectors*, International Atomic Energy Agency, Vienna, pp. 267-278.
- Ryder, L. P., and Svejgaard, A., 1977, Histocompatibility associated diseases, in: *B and T Cells in Immune Recognition* (F. Loor and G. E. Roelants, eds.), John Wiley & Sons, London, pp. 437-557.
- Sacks, D. L., and Askonas, B. A., 1981, Trypanosome induced suppression of antiparasite responses during experimental African trypanosomiasis, *Eur. J. Immunol.* 10:971-974.
- Sacks, D. L., Selkirk, M., Ogilvie, B. M., and Askonas, B. A., 1980, Intrinsic immunosuppressive activity of different trypanosome strains varies with parasite virulence, *Nature* 283:476-478.
- Sato, G. J., Waksal, S. D., and Herzenberg, L. A., 1976, Identification and separation of pre-T cells

- from nu/nu mice: Differentiation by preculture with thymic reticulo-epithelial cells, *Cell. Immunol.* 24:173-188.
- Scheid, M. P., Goldstein, G., and Boyse, E. A., 1975, Differentiation of T cells in nude mice, *Science* 190:1211.
- Schilling, C., 1935, Immunization against trypanosomiasis, *J. Trop. Med. Hyg.* 38:106-108.
- Scott, J. M., Pegram, R. G., Holmes, P. H., Pay, T. W., Knight, D. A., Jennings, F. W., and Urquhart, G. M., 1977, Immunosuppression in bovine trypanosomiasis: Field studies in using foot-and-mouth disease vaccine and clostridial vaccine, *Trop. Anim. Health Prod.* 9:159-165.
- Seed, J. R., 1978, Competition among serologically different clones of *Trypanosoma brucei gambiense* in vivo, *J. Protozool.* 25:526-529.
- Seed, J. R., and Gam, A. A., 1966, Passive immunity to experimental trypanosomiasis, *J. Parasitol.* 52:1134-1140.
- Selkirk, M. E., and Sacks, D. L., 1981, Trypanotolerance in inbred mice: An immunological basis for variation in susceptibility to infection with *Trypanosoma brucei*, *Tropenmed. Parasitol.*, submitted for publication.
- Sollod, A. E., and Frank, G. H., 1979, Bovine trypanosomiasis effect on the immune response of the infected host, *Am. J. Vet. Res.* 40:658-664.
- Soltys, M. A., 1955, Studies on resistance to *T. congolense* developed by Zebu cattle treated prophylactically with Antrycide prosalt in an enzootic area of East Africa, *Ann. Trop. Med. Parasitol.* 49:1-8.
- Ssenyonga, G. S. Z., and Adams, K. M., 1975, The number and morphology of trypanosomes in the blood and lymph of rats infected with *Trypanosoma brucei* and *T. congolense*, *Parasitology* 70:255-261.
- Stephen, L. E., 1966, Observations on the resistance of West African N'Dama and Zebu cattle to trypanosomiasis following challenge by wild *Glossina morsitans* from an early age, *Ann. Trop. Med. Parasitol.* 60:230-246.
- Stewart, J. L., 1951, The West African shorthorn cattle: Their value to Africa as trypanosomiasis-resistant animals, *Vet. Rec.* 63:454-457.
- Tait, A., 1980, Evidence for diploidy and mating in trypanosomes, *Nature* 287:536-538.
- Takayanagi, T., and Enriquez, G. L., 1973, Effects of the IgG and IgM immunoglobulins in *Trypanosoma gambiense* infection in mice, *J. Parasitol.* 59:644-647.
- Takayanagi, T., and Nakatake, Y., 1976, *Trypanosoma gambiense*: Immunity with thymic cell transfer in mice, *Exp. Parasitol.* 39:234-239.
- Takayanagi, T., Nakatake, Y., and Enriquez, G. L., 1974, Attachment and ingestion of *Trypanosoma gambiense* to the rat macrophage by specific antiserum, *J. Parasitol.* 60:336-339.
- Tanner, M., 1980, Studies on the mechanisms supporting the continuous growth of *Trypanosoma (Trypanozoon) brucei* as bloodstream-like form in vitro, *Acta Trop. (Basel)* 37:203-220.
- Tanner, M., Jenni, L., Hecker, H., and Brun, R., 1980, Characterization of *Trypanosoma brucei* isolated from lymph nodes of rats, *Parasitology* 80:383-391.
- Taylor, D. W., and Cross, G. A. M., 1977, The synthesis of a variant-specific antigen by *Trypanosoma brucei* in vitro, *Parasitology* 74:47-60.
- Temu, S. E., and Njogu, A. R., 1975, A review on the use of Berenil in the treatment of human trypanosomiasis, in: *International Scientific Council for Trypanosomiasis Research and Control, Fourteenth Meeting, Dakar, Senegal. OAU/STRC, Nairobi, Publication No. 109*, pp. 47-52.
- Tizard, I. R., and Soltys, M. A., 1970, Macrophage cytophilic antibodies to *Trypanosoma brucei* in rabbits, *Trans. R. Soc. Trop. Med. Hyg.* 65:407-408.
- Tizard, I. R., and Soltys, M. A., 1971, Cell mediated hypersensitivity in rabbits infected with *Trypanosoma brucei* and *Trypanosoma rhodesiense*, *Infect. Immun.* 4:674-677.
- Touré, S. M., Bouffet, P., and Kebe, B., 1975, Comparative frequency of the various species of

- trypanosomes in N'Dama cattle in Senegal, in: *International Scientific Council for Trypanosomiasis Research and Control, Fourteenth Meeting, Dakar, Senegal*, OAU/STRC, Nairobi, Publication No. 109, p. 118.
- Urquhart, G. M., Murray, M., and Jennings, F. W., 1972, The immune response to helminth infection in trypanosome infected animals. *Trans. R. Soc. Trop. Med. Hyg.* 66:342.
- Urquhart, G. M., Murray, M., Jennings, F. W., and Bate, E., 1973, Immunosuppression in *Trypanosoma brucei* infections in rats and mice, *Trans. R. Soc. Trop. Med. Hyg.* 67:528-535.
- Van der Meer, C., Versluijs-Broers, J. A. M., and Oppendoes, F. R., 1979, *Trypanosoma brucei*: Trypanocidal effect of salicylhydroxamic acid plus glycerol in infested rats, *Exp. Parasitol.* 48:126-134.
- Van Hoeve, K., and Cunningham, M. P., 1964, Prophylactic activity of Berenil against trypanosomes in treated cattle, *Vet. Rec.* 76:260.
- Van Saceghem, R., 1938, L'immunisation des bovidés contre la trypanosomiase, *Bull. Soc. Pathol. Exot.* 31:296-298.
- Vickerman, K., 1969, On the surface coat and flagellar adhesion in trypanosomes, *J. Cell. Sci.* 5:163-194.
- Vickerman, K., 1978, Antigenic variation in trypanosomes, *Nature* 273:613-617.
- Vickerman, K., and Luckins, A. G., 1969, Localization of variable antigens in the surface coat of *Trypanosoma brucei* using ferritin conjugated antibody, *Nature* 224:1125-1126.
- Walker, P. J., 1964, Reproduction and heredity in trypanosomes: A critical review dealing mainly with the African species in the mammalian host, *Inter. Rev. Cytol.* 17:51-98.
- Welde, B. T., Hockmeyer, W. T., Kovatch, R. M., and Shogal, M. S., 1979, Immunity in the bovine to *T. congolense* induced by self-cure or chemotherapy, in: *Pathogenicity of Trypanosomes* (G. Losos and A. Chouinard, eds.), International Development Research Center, Ottawa, pp. 82-86.
- Wellhausen, S. R., and Mansfield, J. M., 1979, Lymphocyte function in experimental African trypanosomiasis. II. Splenic suppressor cell activity, *J. Immunol.* 122:818-824.
- Wellhausen, S. R., and Mansfield, J. M., 1980a, Lymphocyte function in experimental African trypanosomiasis. III. Loss of lymph node cell responsiveness, *J. Immunol.* 124:1183-1186.
- Wellhausen, S. R., and Mansfield, J. M., 1980b, Characteristics of splenic suppressor cell-target cell interaction in experimental African trypanosomiasis, *Cell. Immunol.* 54:414-424.
- Wells, E. A., 1972, The importance of mechanical transmission in the epidemiology of nagana: A review, *Trop. Anim. Health Prod.* 4:74-88.
- Whitelaw, D. D., Macaskill, J. A., Holmes, P. H., Jennings, F. W., and Urquhart, G. M., 1980, Genetic resistance to *Trypanosoma congolense* infections in mice, *Infect. Immun.* 27:707-713.
- Wiesenhuber, Von E., 1970, Immunity experiments in Zebu against a *Trypanosoma congolense* infection, *Berl. Munch. Tierarztl. Wochenschr.* 83:218-220.
- Williams, R. O., Marcu, K. B., Young, J. R., Rovis, L., and Williams, S. C., 1978, A characterization of mRNA activities and their sequence complexities in *Trypanosoma brucei*: Partial purification and properties of the VSSA mRNA, *Nucleic Acids Res.* 5:3171-3182.
- Williams, R. O., Young, J. R., and Majiwa, P. A. O., 1979, Genomic rearrangements correlated with antigenic variation in *Trypanosoma brucei*, *Nature* 282:847-849.
- Williams, R. O., Young, J. R., Majiwa, P. A. O., Doyle, J. J., and Shapiro, S. Z., 1980, Analysis of variable antigen gene rearrangements in *Trypanosoma brucei*, *Am. J. Hyg. Trop. Med.* 29:1037-1042.
- Williams, R. O., Young, J. R., Majiwa, P. A. O., Doyle, J. J., and Shapiro, S. Z., 1981, Contextual genomic rearrangements of variable antigen genes in *Trypanosoma brucei*, *Cold Spring Harbor Symp. Quant. Biol.*, 45:945-950.
- Wilson, A. J., 1971, Immunological aspects of bovine trypanosomiasis. III. Patterns in the development of immunity, *Trop. Anim. Health Prod.* 3:14-22.

- Wilson, A. J., and Cunningham, M. P., 1971, Immunological aspects of bovine trypanosomiasis. IV. Patterns in the production of common antibodies, *Trop. Anim. Health Prod.* 3:133–139.
- Wilson, A. J., and Cunningham, M. P., 1972, Immunological aspects of bovine trypanosomiasis. I. Immune response of cattle to infection with *Trypanosoma congolense* and the antigenic variation of the infecting organisms, *Exp. Parasitol.* 32:165–173.
- Wilson, A. J., Paris, J., and Davidson, C. R., 1975a, A study on the development of infections by different trypanosome species in cattle treated regularly with diminazene aceturate, in: *International Scientific Council for Trypanosomiasis Research and Control, Fourteenth Meeting, Dakar, Senegal*, OAU/STRC, Nairobi, Publication No. 109, pp. 90–93.
- Wilson, A. J., Paris, J., and Dar, F. K., 1975b, Maintenance of a herd of breeding cattle in an area of high trypanosome challenge, *Trop. Anim. Health Prod.* 7:63–71.
- Wilson, A. J., Le Roux, J. G., Paris, J., Davidson, C. R., and Gray, A. R., 1975c, Observations on a herd of beef cattle maintained in a tsetse area. I. Assessment of chemotherapy as a method for the control of trypanosomiasis, *Trop. Anim. Health Prod.* 7:187–199.
- Wilson, A. J., Paris, J., Luckins, A. G., Dar, F. K., and Gray, A. R., 1976, Observations on a herd of beef cattle maintained in a tsetse area. II. Assessment of the development of immunity in association with trypanocidal treatment, *Trop. Anim. Health Prod.* 8:1–12.
- Yamauchi, K., Green, D. R., Eardley, D. D., Murphy, D. B., and Gershon, R. K., 1981, Immunoregulatory circuits that modulate responsiveness to suppressor cell signals: The failure of B10 mice to respond to suppressor factors can be overcome by quenching the contrasuppressor circuit, *J. Exp. Med.* 153:1547–1561.
- Zahalsky, A. C., and Weinberg, R. L., 1976, Immunity to monomorphic *Trypanosoma brucei*: Humoral response, *J. Parasitol.* 62:15–19.

Shigellosis

GERALD KEUSCH

1. INTRODUCTION

Infections caused by the genus *Shigella* occur in every corner of the globe. The organisms are highly host adapted to the human species and a few other higher primates, and it appears that wherever these hosts exist, there too *Shigella* infections will occur, whether in the Arctic Circle or at the equator. However, the incidence and prevalence of infection, and its severity, are much greater in populations living in the developing world, undoubtedly in part because of a lack of adequate environmental sanitation and the high prevalence of concomitant malnutrition. In this special sense, shigellosis is a tropical disease, and this chapter attempts to address those features of the host, microorganism, and environment that combine to make this so.

2. MICROBIOLOGY

2.1. General Considerations

The shigellas are classified in the family Enterobacteriaceae, in the tribe Eschericheae. In many respects they are bacterial brothers to the common *E. coli*; they are oxidase negative, rod-shaped, gram-negative bacteria that ferment glucose but do not produce gas. However, the shigellas are nonmotile, and they do not ferment lactose, and in these properties, as well as in their ability to invade epithelial cells, they are distinct from the majority of *E. coli* (Keusch, 1979). Actually, a few shigellalike serotypes of *E. coli* are invasive, and these

Table I
Differentiation of *Shigellas* and *E. coli*

Characteristic	Microorganism				
	<i>S. dysenteriae</i>	<i>S. flexneri</i>	<i>S. boydii</i>	<i>S. sonnei</i>	<i>E. coli</i>
Utilization of					
Glucose	+	+	+	+	+
Lactose	—	—	—	— (late +)	+
Mannitol	—	+	+	+	+
Lysine decarboxylase	—	—	—	—	+
Ornithine decarboxylase	—	—	—	+	+
Motility	—	—	—	—	+
Gas from glucose	—	—	—	—	+
Serogroup	A	B	C	D	N/A

strains produce shigellalike disease (DuPont *et al.*, 1971). The latter organisms (0:28ac, 0:112, 0:124, 0:136, 0:143, 0:144, 0:152, 0:164) may be distinguished from the true shigellas on the basis of a positive lysine decarboxylase reaction. There are four shigella species which are differentiated on the basis of biochemical and serologic differences: *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei* (Table I).

2.2. Isolation and Identification

Isolation and identification of the shigellas in the clinical laboratory depend first and foremost on detection of their inability to ferment lactose (although *S. sonnei* is capable of performing this reaction, but only after several days in culture). In practice, this is accomplished through the use of selective and differential media which are designed to accomplish two things: (1) suppression of other components of the fecal flora by inclusion of inhibitory substances such as bile salts and (2) dramatically spotlight non-lactose-fermenting colonies through inclusion of an indicator dye system in the medium. This allows the clinical microbiologist to easily select non-lactose-fermenting colonies for further investigation and identification. Many different formulas have been developed for such selective differential media, including MacConkey's, xylose-lysine-deoxycholate (XLD), Hektoen enteric (HE), tergitol-7-tetraphenyl tetrazolium (T-7-T), and *Salmonella-Shigella* (SS). Although all of these media support growth of salmonella, some may be suppressive to the growth of shigellas. MacConkey's is probably the least inhibitory, whereas SS agar can be highly inhibitory to certain shigella species, especially strains bearing R-factors (Mata *et al.*, 1970; Rahaman *et al.*, 1975a; Yoshikawa, 1972). It is therefore highly recommended that more than one medium be used in order to maximize

the yield of positive cultures. The number of positive cultures can also be increased by preincubating a sample in a selective liquid medium such as Hajna's gram-negative broth (Keusch, 1979). Ironically, in spite of the fierce clinical disease they produce, the shigellas are not hardy bacteria outside the human host and, in fact, are somewhat dainty. If culture samples cannot be plated and incubated rapidly, it is best to employ a holding medium such as Carey Blair transport medium. The yield can also be increased by attention to the sample submitted for microbiological analysis (Ewing and Martin, 1974). Stool swabs are much less likely to be positive than culture of a stool specimen itself. In addition, selection of mucoid or exudative portion of the sample will also increase the yield of positives. With these few technical details in mind, it is not difficult for even a simply equipped laboratory to isolate, identify, and speciate the shigellas.

3. PATHOGENESIS

Two features of the genus are currently implicated in pathogenesis of the disease they cause. These are the capacity to invade intestinal epithelial cells and to produce cell-free protein toxins that may mediate the pathophysiology of the infection (Keusch, 1979).

3.1. Tissue Invasion

Many studies have demonstrated a close correlation between the ability of shigellas to invade epithelial cells and their ability to produce disease (LaBrec *et al.*, 1964; Formal *et al.*, 1965a, 1966; Gemski *et al.*, 1972a; Levine *et al.*, 1973). Virulent strains are always found to be invasive, a property that may be demonstrated in *in vitro* cell culture or *in vivo* in the cornea of experimental animals or in the intestinal tract of experimental animals or in human patients with shigellosis. The correlation is also shown by selection of noninvasive strains which are then found to be avirulent. Selection of such strains may be accomplished by picking colonial variants of the invasive virulent bacterium that arise during culture on artificial media. The various shigella species undergo a spontaneous colonial mutation from translucent to opaque; investigation of these colonial variants demonstrates that the change is associated with loss of invasive capacity and virulence (DuPont *et al.*, 1972; Gemski *et al.*, 1972a; Levine *et al.*, 1973). Whereas translucent colonies give rise to opaque variants, the reverse does not seem to be true.

Recent evidence suggests that the translucent-to-opaque transformation is associated with loss of a large 1.2–1.45 megadalton plasmid in both *S. sonnei* and *S. flexneri* (Kopecko *et al.*, 1980). Alterations in the bacterial cell surface,

such as charge and detergent sensitivity, as well as enzymatic changes, particularly of glycerol kinase, accompany the phenotypic transformation (Corwin *et al.*, 1971, 1972; Kim and Corwin, 1974). Hybridization studies in which chromosomal segments from *E. coli* are inserted into *Shigella* species support the role of invasion in pathogenesis and, in addition, demonstrate the genetic complexity of the phenomenon (Formal *et al.*, 1965b). Insertion of *E. coli* 0-25 serotype genes into *Shigella flexneri* does not alter the invasiveness of the hybrid (Gemski *et al.*, 1972b). However, in both *Shigella flexneri* and *E. coli* 0-25, the immunodominant sugar of the O-somatic antigen, rhamnose, is the same. In contrast, insertion of *E. coli* 0-8 somatic antigen into *S. flexneri* simultaneously changes the dominant sugar composition of the O-antigen to mannose and renders the organism noninvasive and avirulent. Two chromosomal loci are associated with these changes, the Kpcr and xylose-rhamnose regions. The specific nature or mechanism of the invasive process *per se* is, however, unknown.

3.2. Toxin Production

Just 2 years after the organism was first isolated and described by Shiga in 1898 during a massive epidemic of dysentery in Japan (over 90,000 cases and over 20,000 deaths), Flexner (1900) suggested that the shigellas were toxigenic. However, the effects noted by Flexner in his experiments were probably caused by lipopolysaccharide endotoxin and not by the distinct protein toxin produced by the genus. Credit for the discovery of this toxin in filtrates of *S. dysenteriae* 1 is usually given to Conradi (1903) who demonstrated that parenteral administration of bacterial filtrates into rabbits resulted in a delayed onset paresis and then paralysis of the limbs, followed by death. Because of these clinical manifestations, the toxic principle has ever since been called neurotoxin. During the next 20 years, a number of investigations demonstrated that neurotoxin was a protein, distinct from the endotoxin, without significant effects on the gastrointestinal tract when given parenterally to experimental animals (Keusch *et al.*, 1982). Other species of *Shigella* could not be shown to produce the same neurotoxin. However, large intravenous doses of endotoxin from the different *Shigella* species produced hemorrhagic lesions of the gut and were highly lethal, and for this reason some workers began to refer to the endotoxin as an enterotoxin. In view of the fact that all bacterial lipopolysaccharide (LPS) endotoxins share similar properties, it seemed difficult to explain why the shigellas were so virulent and the other organisms were not. Virulence could not be simply attributed to neurotoxin either, because it was apparently produced only by *Shigella dysenteriae* 1, whereas all *Shigella* species produced a similar clinical disease. Thus, neither LPS nor neurotoxin seemed likely to be involved in the pathogenesis of shigellosis.

In the past decade, however, it has been shown that all species of shigella in

fact produce a protein toxin (Keusch *et al.*, 1972a; Keusch and Jacewicz, 1977a). Furthermore, this toxin exerts potent effects on the gastrointestinal tract when applied directly to the mucosal surface rather than via a parenteral route. This toxicity is currently called enterotoxin (Keusch *et al.*, 1972a,b), but it should be made absolutely clear that it is distinct from LPS which does not have similar activity in the gut lumen. In both rabbit jejunum and ileum, protein enterotoxin preparations, but not LPS, induce the net secretion of water and electrolytes (Keusch *et al.*, 1972a; Steinberg *et al.*, 1975), a property in common with the now classical enterotoxins of *Vibrio cholerae* and toxigenic *E. coli* (Grady and Keusch, 1971). In addition, striking histological abnormalities are produced in the ileum (Keusch *et al.*, 1972b) but not jejunum. These consist of epithelial cell death, microulcer formation, and a marked inflammatory infiltrate in the lamina propria. Similar histological features are observed in the colon of the patient affected with shigellosis as well (Keusch, 1979). Purified cell-free toxin preparations having this activity are, in addition, found to be neurotoxic to mice or rabbits and to be lethal to certain cell types in *in vitro* culture, an activity now described as cytotoxin (Keusch and Jacewicz, 1975). Current evidence suggests that one toxin exerts all three biological effects, but this is not yet certain.

3.3. Mechanism of Action of Toxin(s)

The mechanism of action of the enterotoxin in the gastrointestinal tract is not characterized. In the jejunum it is known to produce a secretory response similar to that of cholera toxin (Steinberg *et al.*, 1975), but the evidence to date that it shares the same mechanism, namely, activation of adenylate cyclase, is controversial (Charney *et al.*, 1976; Flores *et al.*, 1974). Much more is known about the cytotoxin activity, particularly in cell culture systems employing HeLa cells. These studies demonstrate that toxin first binds to the HeLa cell surface via a glycoprotein receptor containing oligomeric $\beta 1 \rightarrow 4$ N-acetyl-D-glucosamine (Keusch and Jacewicz, 1977b). Following binding, toxin is transported to the cell interior and therein inhibits protein synthesis (Keusch, 1979; Keusch, 1981).

Inhibition of protein synthesis induced by cytotoxin has been demonstrated in intact cells in culture and in *in vitro* cell-free systems derived from both mammalian and bacterial cells (Brown *et al.*, 1980; Olenick and Wolfe, 1980; Reisbig *et al.*, 1981). The mechanism by which this occurs is under active investigation, but Brown *et al.* (1980) believe the toxin inhibits elongation of peptides being made on ribosomes.

Attempts to produce nontoxigenic organisms in order to further unravel the role of toxin in pathogenesis have not been successful. Gemski *et al.* (1972a) obtained an invasive "nontoxigenic" *Shigella dysenteriae* 1 by mutation and selection for chlorate resistance. This strain has actually proven to be a hypotox-

igenic regulatory mutant that produces 100-fold less toxin *in vitro* but remains virulent (Keusch *et al.*, 1976). Human volunteers infected with such strains develop clinical shigellosis (Levine *et al.*, 1973) but also demonstrate development of toxin-neutralizing antibody in serum (Keusch *et al.*, 1976). At the present time, then, it would appear that both the enterotoxin and cytotoxin activities are important factors in causation of clinical shigellosis (Keusch, 1979, 1981; Keusch *et al.*, 1982). This is discussed further in Section 6.

4. CLINICAL MANIFESTATIONS

4.1. Intestinal Disease

4.1.1. Dysentery

Shigella infections are confined to the gastrointestinal tract in most patients, and the major manifestations of the disease are, accordingly, intestinal. *Shigella* species are well-known causes of the dysentery syndrome, a triad of symptoms and signs including cramping abdominal pain, tenesmus (intense spasms of pain during defecation), and frequent passage of bloody mucoid stools (Figure 1) occurring 30–40 times a day (Keusch, 1979). The association of shigella species with this clinical syndrome has led to the designation bacillary dysentery, to distinguish it from the dysentery caused by *E. histolytica*. Sigmoidoscopy of patients with bacillary dysentery reveals hemorrhages in the mucosa, mucus secretion, mucosal friability, and microulcers with exudate. Colonic biopsy is consistent with this gross pathology and shows focal epithelial cell damage, mucosal sloughing, and intense bleeding, and inflammation in the lamina propria (Figure 2). Giemsa or tissue gram stains show the presence of numerous microorganisms within colonic intestinal epithelial cells (Figure 3) and, to a lesser extent, free in the lamina propria (Keusch, 1979), and indeed, the colon appears to be the target organ for the invasive property of the microorganism (Rout *et al.*, 1975). The clinical dysentery syndrome and the gross and microscopic pathology associated with it are thus the result of an invasive bacterial colitis.

Ingestion of noninvasive colonial mutants or *Shigella*–*E. coli* hybrids produces none of this pathology and, of course, is not associated with production of symptoms (Formal *et al.*, 1971).

4.1.2. Diarrhea

In the past decade, however, it has become widely appreciated that dysentery is not the only intestinal manifestation of shigellosis, nor indeed is it the

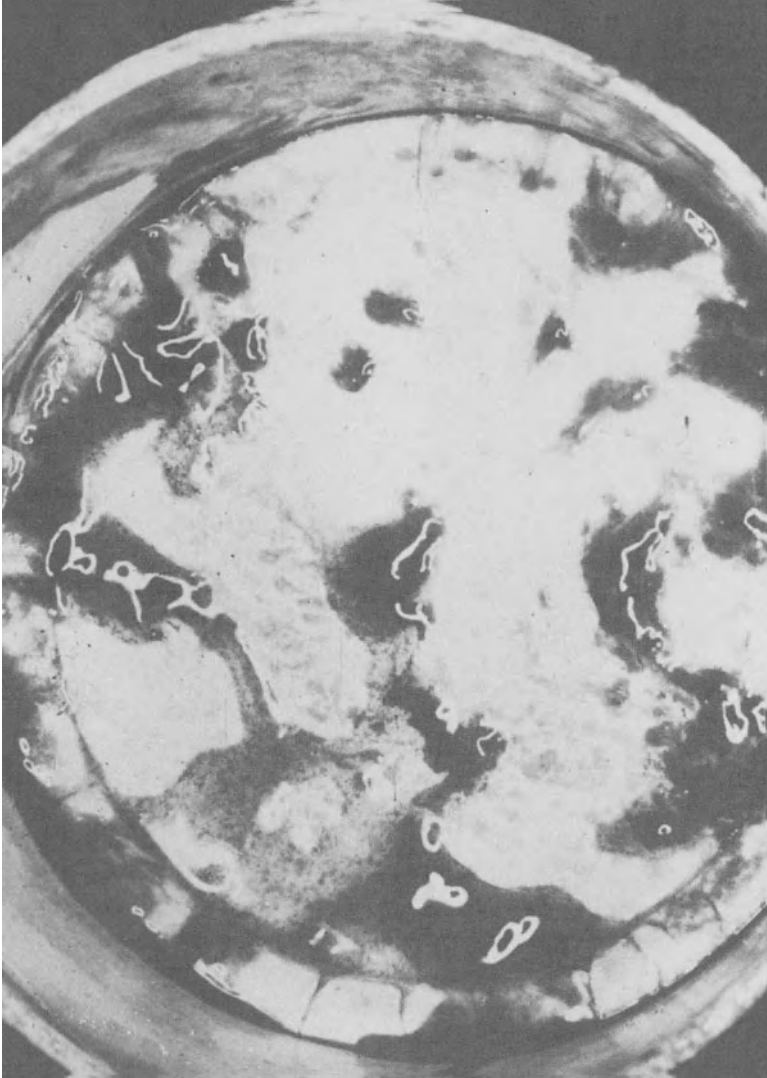


Figure 1. The typical bacillary dysentery stool. Even in this black-and-white photograph, the blood and the mucoid discharge which is the clinical *sine qua non* of dysentery can be easily appreciated. The scant quantity of stool is also a prominent feature of the disease (courtesy of Myron M. Levine).

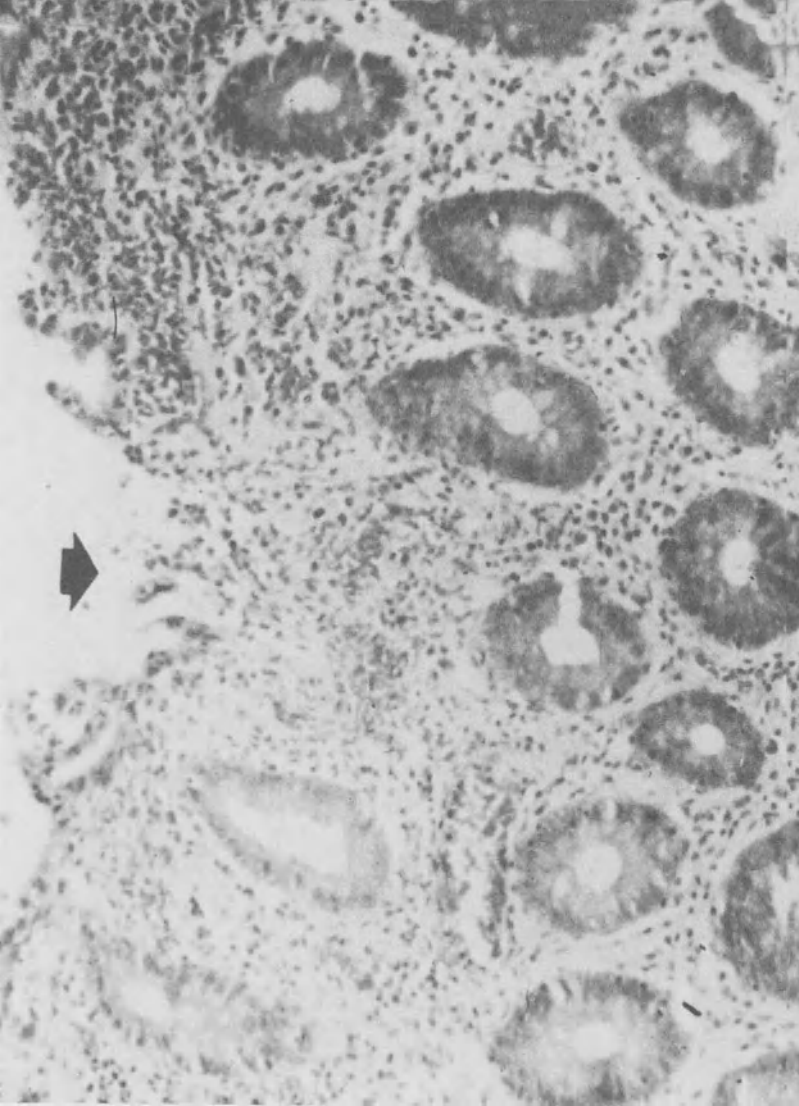


Figure 2. Colonic biopsy from a patient with acute shigellosis. Of note is the marked inflammatory infiltrate in the lamina propria and the microulcer (arrow) resulting from focal death and sloughing of the colonic epithelium (hematoxylin and eosin).

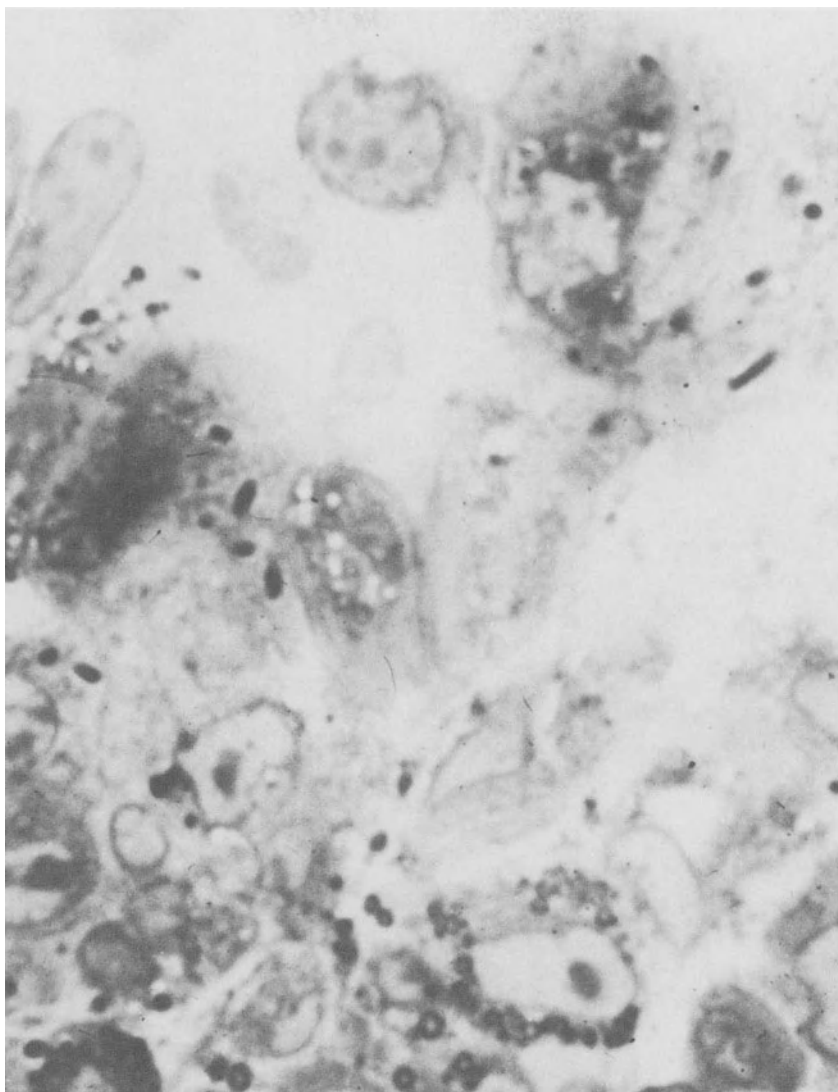


Figure 3. High-power photomicrograph from the region of a colonic ulcer in a patient with *Shigella* dysentery. The invading microorganisms are visible as dark-staining bacilli within the cells. Also evident is the disarray of the epithelial cell lining and the abnormal appearance of the individual infected cells which will undoubtedly ultimately succumb to the intracellular infection and thus enlarge the ulcerations (Giemsa stain) (courtesy of Samuel B. Formal).

most common (DuPont *et al.*, 1969). In fact, a watery diarrhea syndrome occurs more frequently and is the initial manifestation of illness even in those who develop the dysentery syndrome itself. Microscopic examination of the diarrheal stool shows large numbers of inflammatory cells (Figure 4) (Harris *et al.*, 1972; Korzeniowski *et al.*, 1979; Pickering *et al.*, 1977). The nature of the manifestations depends to some extent on the species of the infecting *Shigella* organism, with *S. dysenteriae* 1 more likely to produce dysentery, *S. sonnei* more likely to produce watery diarrhea alone, and *S. flexneri* somewhere in the middle (Keusch, 1981). In the case of *S. dysenteriae* 1, clinical manifestations may evolve so rapidly from watery diarrhea to dysentery that the diarrhea phase may be overlooked in the telescoped clinical picture in the individual patient (Mata and Castro, 1974). Recent studies in the rhesus monkey orally infected with *S. flexneri* 2a have shed light on the mechanism and the origin of the watery diarrhea (Rout *et al.*, 1975). In all animals exhibiting this clinical manifestation, the proximal small bowel is found to be in a net secretory state for water and electrolytes. This occurs in the absence of bacterial invasion in the jejunum, although many microorganisms are present in the lumen of the bowel.

In human experimental volunteer studies, infection with 10,000 *Shigella flexneri* 2a produces no disease in 25%, fever as the only manifestation of disease in another 25%, fever accompanied by watery diarrhea in 25%, and fever and watery diarrhea progressing to the dysentery syndrome in the remaining 25% (DuPont *et al.*, 1969). With *S. dysenteriae* 1, as few as 200 organisms produce diarrhea and dysenteric manifestations in a quarter of those infected (Levine *et al.*, 1973).

4.2. Fever

Fever is a constant accompaniment of shigellosis and, in fact, in young children may lead to the development of typical febrile convulsions (Keusch, 1979). In addition, in young children the initial clinical manifestations may be fever, headache, and stiff neck, suggesting the possibility of acute meningitis (Barrett-Connor and Connor, 1970). By lumbar puncture, however, the cerebral spinal fluid is found to be normal and sterile, and the true nature of the disease is revealed when the intestinal symptoms begin. In both children and adults, the disease may be initiated by respiratory symptoms, such as cough, sometimes accompanied by rales on physical examination, and even by infiltrates on the chest X-ray. But rather than progressing to a true pneumonia, these symptoms and signs abate as the gastrointestinal illness becomes evident, and the respiratory manifestations subside without specific treatment. Nevertheless, an occasional patient with bacterial invasion of the central nervous system or of the lung has been recorded (Barrett-Connor and Connor, 1970).

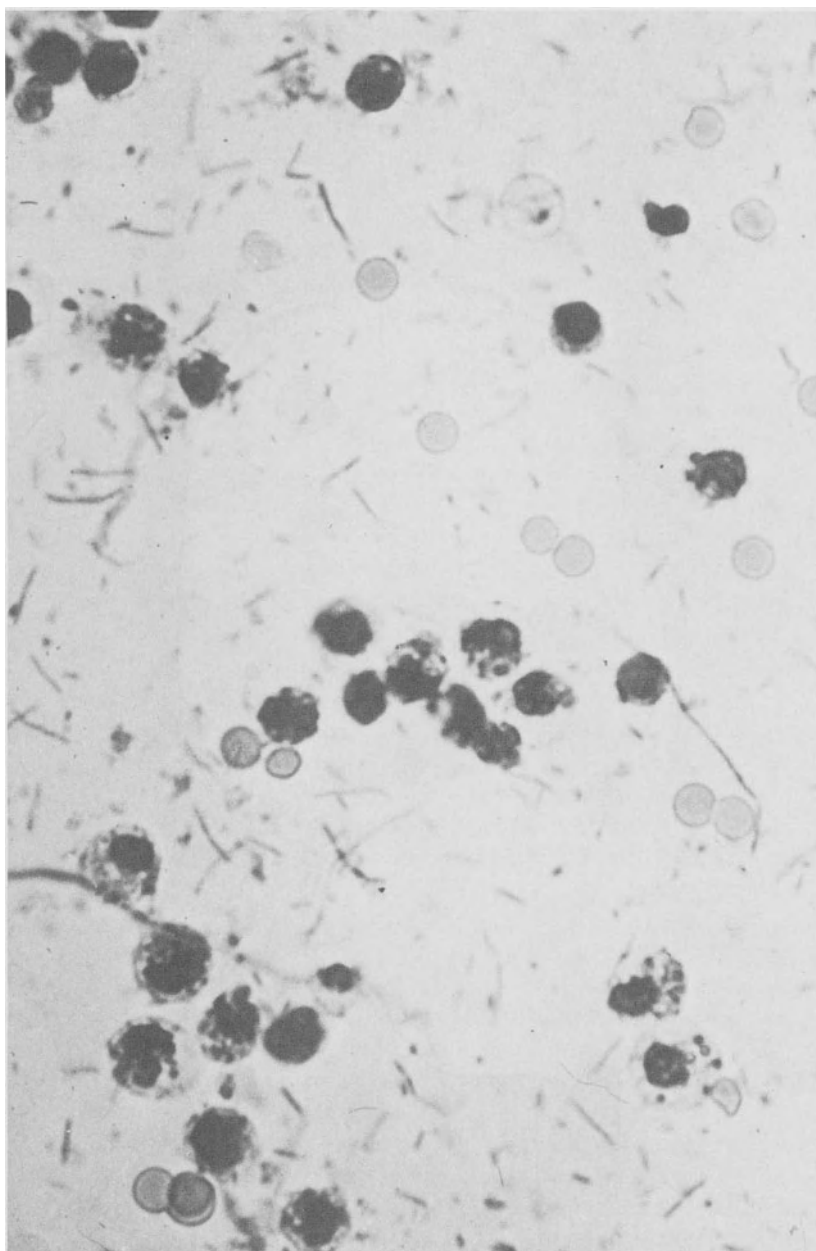


Figure 4. Low-power micrograph of the watery diarrheal stool of a patient with *Shigella* diarrhea. Many leukocytes and erythrocytes are present in addition to the large number of bacteria. Such an exudative stool is a characteristic, but not pathognomonic, finding in shigellosis (courtesy of Myron M. Levine).

4.3. Extraintestinal Manifestations

A number of additional extraintestinal manifestations of shigellosis have been described. Rose spots similar to skin lesions associated with typhoid fever have been seen on occasion in patients with *Shigella* infections (Barrett-Connor and Connor, 1969). A few patients have been described with *Shigella* infection of skin wounds or of the cornea, the human equivalent of the Sereny test (Roper, 1979). During the recent outbreaks of Shiga bacillus infection in Central America and in Bangladesh, patients with leukemoid responses in the peripheral blood, with white counts greater than 50,000, have been seen with some frequency (Castro *et al.*, 1974; Rahaman *et al.*, 1975b). In some individuals, this has progressed to microangiopathic hemolytic anemia, with or without evidence of disseminated intravascular coagulation and uremia (hemolytic-uremic syndrome). These manifestations have generally occurred 4–7 days after the onset of illness, at a time when the patient is usually improving, and they have not been seen with infection caused by other species of *Shigella* (Koster *et al.*, 1978).

Reactive polyarthritis, arthritis of large joints, with or without other manifestations of Reiter's syndrome (including conjunctivitis and urethritis) has been observed within a few weeks of the intestinal phase of the disease (Keusch, 1979; Noer, 1966). Manifestations of Reiter's syndrome have occurred in any order and, in general, clear without residua. Reactive polyarthritis occurring in patients with histocompatibility antigen HLA-B27 may be particularly severe and prolonged and often results in destructive joint lesions (Calin and Fries, 1976). The joint fluids are sterile, although a few patients with acute septic arthritis or osteomyelitis have been reported (Keusch, 1979).

Shigella vulvovaginitis has been described in some 30 patients to date, the majority young girls less than 15 years of age (Murphy, 1979). An occasional patient will present with *Shigella* urinary tract infection, usually as an asymptomatic bacteriuria (Jao and Jackson, 1963).

In spite of the evidence of mucosal invasion by the organism and the myriad of systemic and disseminated clinical manifestations, as well as the development of serum antibodies to the somatic O-antigen of the organism (Cáceres and Mata, 1974) and neutralizing antibody to the cytotoxin (Keusch and Jacewicz, 1973), proven bacteremia is unusual. Although more than 100 cases have been described in the literature, bacteremia is the exception rather than the rule in patients with shigellosis (Keusch, 1979; Koshi *et al.*, 1979).

The reasons for the general failure of the organism to infect the bloodstream are not known but might be related to the observation of Reed and Albright (1974) who demonstrated that most *Shigella* isolates are highly sensitive to the complement-mediated serum bactericidal reaction. Thus, the organism would be rapidly cleared from the blood if it managed to reach the circulation. Recently, Moore *et al.* (1980) have shown the presence of an antibody in normal serum to an enterochelinlike siderophore on the surface of *Shigella flexneri* that exerts a

bacteriostatic effect by preventing acquisition of needed iron by the microorganism. It is possible that such antibody activity also affects the ability of the organism to survive in the bloodstream.

4.4. Malnutrition

A recent review by Scragg *et al.* (1978) of 948 patients with shigellosis in Durban, South Africa, 6 days to 12 years of age (median age of 17 months) revealed a rather high mortality rate of 27%. Among the 254 deaths, 226 (89%) occurred in severely malnourished subjects. Blood cultures were obtained in 569 patients, and 11 were positive, nine occurring in malnourished individuals. Deaths in the bacteremic group were restricted to the malnourished; five of the nine subjects (55.6%) succumbed. Mortality was thus clearly related to both malnutrition and septicemic spread of the organisms and is certainly a direct consequence of the debilitated state of these patients.

4.4.1. Polymicrobial Sepsis in Shigellosis

A number of patients also have been reported with shigellosis and bacteremia caused by another organism. Barrett-Connor and Connor (1970) have reported three cases of concomitant pneumococcal bacteremia, two of which occurred in patients with evidence of pneumonia. Neglia *et al.* (1976) found secondary *Klebsiella* bacteremia in two patients with *S. sonnei* dysentery, and Haltalin and Nelson (1965) described two patients with *Enterobacter aerogenes* sepsis and another from whom both *E. aerogenes* and *E. coli* were recovered. Likewise, Scragg *et al.* (1978) had one patient with combined *Shigella* and *Salmonella* sepsis. The probable entry for the gram-negative bacteria is the damaged colonic mucosa.

4.5. Diagnosis

Diagnosis depends on isolation of the organism from stool. This is not difficult if an appropriate sample (stool in preference to a swab) can be cultured quickly or, if not, is placed in a holding or transport medium. The presence of fecal leukocytes is a good clue but can be caused by other agents as well (Harris *et al.*, 1972; Korzeniowski *et al.*, 1979; Pickering *et al.*, 1977).

5. EPIDEMIOLOGY

5.1. Inoculum Size

An important determinant in the epidemiology of *Shigella* infections is the ability of the microorganism to cause disease when ingested in very small inocu-

la. With virulent *S. dysenteriae* 1, as few as 10–100 viable organisms produce symptomatic disease in about one-fifth of patients (Levine *et al.*, 1973). *Shigella flexneri* 2a or *S. sonnei* will cause disease in two-thirds to three-fourths of patients at a dose of 10,000 microorganisms (DuPont *et al.*, 1969). Under comparable circumstances (that is, without concomitant administration of sodium bicarbonate), more than 100,000 *Salmonella* gastroenteritis strains (Turnbull, 1979) are required to initiate disease and as many as 100 million to 1 billion toxigenic *E. coli* or *V. cholerae* are needed (Music *et al.*, 1971; Satterwhite *et al.*, 1978). This ability to cause disease through ingestion of small inocula is the basis for the ease in person-to-person transmission of *Shigella* infection but not cholera. This is perhaps fortunate for the microorganism, because it does not survive well in the environment (Keusch, 1982).

5.2. Transmission

Person-to-person transmission of infection also accounts for the frequency of household cases in families following introduction of an infection, usually by a preschool or school-age child (Keusch, 1982; Weissman *et al.*, 1974). The secondary household infection rate among children is around 40%, and the rate of secondary cases among adults is 10–15% (Baine *et al.*, 1975; Hardy and Watt, 1945; Thomas and Tillett, 1973). In a more recent study (Thomas *et al.*, 1972) of two related outbreaks of *S. sonnei* in neighboring North London schools caused by two distinct organisms, one kanamycin sensitive and the other kanamycin resistant, 65% of households and 36% of contacts were infected. The overall incidence in exposed children was 51% (45/89) and was no different for the two strains. In adults, on the other hand, 10% (3/31) were infected with the kanamycin-sensitive strains, whereas 40% (23/76) developed secondary infection by the resistant strain. The incidence of symptomatic secondary infection was high (75%), and the median duration of excretion of the organism was 24 days.

Person-to-person contact is not the only means of spread of shigellosis, and instances of transmission by water or food have been well described in the past (Black *et al.*, 1978; Donadio and Gangarosa, 1969). Community-wide epidemics have occurred when the water supply has been contaminated by raw sewage, and recently a number of outbreaks have been reported aboard cruise ships because of contamination of the water (Black *et al.*, 1978; Drachman *et al.*, 1960; Merson *et al.*, 1975). Hospital epidemics have also been described in which the vehicle has been milk or ice cream (Black *et al.*, 1978). Occasional cases have also been traced to infected monkeys, either kept as pets within households or in institutions such as zoos or research facilities (Cooper and Needham, 1976; Fox, 1975).

5.3. Incidence

Throughout the world the major target population for *Shigella* infection is the pediatric age group (Keusch, 1982). Over two-thirds of the isolates in the United States come from children under the age of 10, the majority, in fact, from children under the age of 5 (Rosenberg *et al.*, 1976). It is difficult to determine the true incidence of shigellosis because there is no good estimate of the number of subclinical or mild infections. A significant proportion of the *S. sonnei* disease is so mild that a physician is not consulted, and cultures for diagnosis are not performed. The best available figure is based on the number of isolates reported to the Centers for Disease Control and the estimated population size of the United States. Using these data, an incidence of 5.3 infections per 100,000 population can be calculated (Keusch, 1982). Considering only children, the rate rises to approximately 18 per 100,000 in children under 10 and 27 per 100,000 in infants and children under the age of 5. These figures are quite different in the developing world.

Prospective longitudinal studies in Guatemala, Egypt, and India demonstrate the incidence of shigellosis to be approximately 200,000 cases per 100,000 individuals per year in those 1 to 4 years of age (Feldman *et al.*, 1970; Floyd *et al.*, 1956; Mata, 1978). It is not certain if this enormous incidence rate represents the effect of a greater density of organisms in the environment because of the lack of clean water supplies for drinking and personal hygiene and the lack of facilities for sanitary disposal of fecal waste or whether the frequent malnutrition in such populations enhances susceptibility as well. It is likely that a combination of these factors is involved in producing such high infection rates. The incidence of shigellosis in older children and adults in the developing world is considerably less, at least for the endemic species of the organism, *S. flexneri* and *S. sonnei*. However, when a new strain is introduced into the community, such as the recent epidemics of *S. dysenteriae* 1 in Central America, Mexico, and Bangladesh (Gangarosa *et al.*, 1970; Rahaman *et al.*, 1975c), the older child and adult population are highly susceptible as well as the young. Similar epidemiological patterns of infection are observed with other endemic and epidemic microorganisms and clearly reflect the acquired immunity effect of frequent contact with endemic strains, whereas the entire population is susceptible to organisms uncommonly encountered.

In contrast, breast-fed children in the developing world may also be infected with shigellas but appear to be relatively protected against clinical manifestations of the disease (Mata *et al.*, 1969), in part because the intestinal flora of the nursing infant exerts a suppressive effect on the *in vivo* growth of shigellas. It is also quite possible that the surface of the intestinal epithelial cells or the nature of the secreted glycoproteins in the breast-fed infant preclude the cell-to-cell interaction necessary for initiation of infection. Phenotypic alterations in the cell

surface are known to mediate the resistance of certain animals to specific enteric pathogens (Gibbons *et al.*, 1977; Sellwood *et al.*, 1975), and this may well be true of *Shigella* infections too. This speculation is certain to be a topic of investigation in the years ahead. This relative protection in breast-fed infants is quite distinct from the highly virulent neonatal infections with shigellas described in the United States which, although rare, are generally serious, with frequent invasion of the bloodstream and high mortality rates (Haltalin, 1967). These infants are usually formula fed and do not have the typical *Bifidobacterium bifidus* flora of the breast-fed child.

Is the relatively low incidence of shigellosis in the United States, even in the under-5-year-old group, the expected situation, or is the situation in developing countries the norm? To highlight the difference even more, it should be pointed out that even if 99% of the *Shigella* infections in the pediatric age group in the United States went undetected, the incidence in the developing world would still be 25 times greater than that observed in the United States (Keusch, 1982). However, there are circumstances in which populations of the United States resemble those in the developing world. For example, shigellosis occurs with much greater frequency in children in day care nurseries and in institutions for the mentally retarded. Prospective studies in the latter group demonstrate an incidence of about 35,000 per 100,000 per year as a minimum figure (DuPont *et al.*, 1970). The epidemiologic association of bacillary dysentery among the inmates of institutions has long been noted and described as an entity, asylum dysentery. This association clearly shows that crowding and poor environmental sanitation of feces will determine incidence of shigellosis. The rates can clearly be driven down by investments in social development and environmental sanitation.

5.4. Host Factors

Are host factors also of importance? Patients with active shigellosis excrete large numbers (10^5 – 10^8) of the pathogen per gram of stool. Asymptomatic carriers often excrete as many as 10^3 organisms per gram of stool (Dale and Mata, 1968). Given the small number of organisms needed to cause infection and disease, it is not surprising that dissemination of infection occurs under circumstances in which personal hygiene is poorly maintained. In the otherwise healthy, prolonged convalescent carriage of shigellas is not common (DuPont *et al.*, 1969). The organism is rarely detected 2 months after infection. This is not true in children in the developing world where carriage for as long as a year has been described with some degree of frequency (Mata *et al.*, 1966). The more virulent strains of *Shigella*, *dysenteriae* 1 and *flexneri*, tend to persist for longer periods of time in such patients than does *S. sonnei* (Mata, 1978), although it is difficult at times to separate persistent infection from acquisition of new infection.

The association of shigellosis and protein–energy malnutrition (PEM) is particularly strong. Deterioration of nutritional state is frequently noted during and after acute shigellosis (Mata, 1978). This may in part be because of the frequent association of fever and the concomitant metabolic alterations that occur acutely or because of the tendency of the infection to be persistent or recurrent, but in either case a bout of shigellosis usually initiates a downward inflection of the growth curve of a child. When this is followed by a series of other infections, catch-up growth can never occur, and the synergistic effects of infection and deteriorating nutritional state can precipitate acute PEM.

Since the introduction of oral rehydration therapy for the acute watery diarrheas caused by *Vibrio cholerae*, toxigenic *E. coli*, and rotavirus, the mortality rate for these infections in the developing world has decreased, unmasking the persistent significant mortality associated with shigellosis and dysentery (Chen *et al.*, 1980).

Gastric acid does not seem to be as important a factor in determining susceptibility to shigellosis as it is for salmonellosis or cholera. The reasons are not clear but may in part be related to the small inoculum needed to induce disease, which is in turn related to the invasive properties of the organism and characteristics of the bacterial cell surface. Although *Shigella flexneri* 2a is acid susceptible *in vitro*, it does not seem to behave that way *in vivo*. Experimental human infection is readily accomplished with a few hundred organisms suspended in 30–45 ml of milk (DuPont *et al.*, 1969; Levine *et al.*, 1973) which will hardly buffer the gastric acidity at all. In contrast, in order to obtain colonization by attenuated noninvasive variants, the inoculum must be preceded by a large dose of bicarbonate (DuPont *et al.*, 1972). The basis for the difference in the *in vitro* and *in vivo* results with the virulent *Shigella* strains is not obvious.

6. CLINICAL–PATHOLOGICAL CORRELATIONS

6.1. Target Organs

Current understanding of the pathogenesis of shigellosis allows reasonable speculation about the pathophysiology of the clinical manifestations of the intestinal disease. The complete clinical picture is an initial watery diarrhea which progresses to the dysentery syndrome (Keusch, 1979). The more highly virulent the organism, the more rapid is the progression to dysentery. Indeed, in the case of *S. dysenteriae* 1 infection, the clinical course is so telescoped that the dysentery overshadows the diarrhea, and the latter is easily overlooked as a distinct phase of illness. Perhaps this is why most textbooks fail to even mention diarrhea as the usual presenting feature of infection or as the sole intestinal manifestation.

6.2. Diarrhea

Experimental rhesus monkey shigellosis clearly documents that the diarrhea and dysentery result from involvement of widely separated segments of bowel, the proximal small intestine and the colon (Rout *et al.*, 1975). Bacterial proliferation is observed in the small intestinal lumen without evidence of mucosal invasion. At the site of luminal proliferation, there is a brisk net secretion of isotonic fluid. Because direct infection of cecum (which bypasses the small bowel and precludes local multiplication) does not result in transport abnormalities in jejunum, it seems quite certain that local bacterial growth is a necessary prerequisite for this to occur. These events are reproduced by inoculation of cell-free enterotoxin in rabbit jejunum, which provides the best evidence at the present time that the watery diarrhea is a toxin-mediated secretory process.

6.3. Dysentery

Dysentery is the clinical consequence of an acute mucosal inflammatory process in the colon, including death and sloughing of infected epithelial cells. Invasion of epithelium and intracellular bacterial multiplication are a necessary part of the pathogenesis, as shown by the lack of virulence of variant strains unable to accomplish these tasks. The destructive mucosal lesion may release prostaglandins locally, resulting in an enhancement of symptomatology, because administration of indomethacin will partially reverse the gut response in a model system in rabbit ileum which mimics events in the colon (Gots *et al.*, 1974). The underlying lesion may be caused by the cytolethal toxin which can be produced intracellularly by invading microorganisms (Hale and Formal, 1981). The biochemical effect of the cytotoxin is a profound inhibition of protein synthesis which is incompatible with cell survival (Keusch, 1979; Olsnes and Eiklid, 1980). Motor abnormalities of gut which cause the intense abdominal cramps and tenesmus so prominent in dysentery (Keusch, 1979) can be caused either by the destructive inflammation or possibly by a direct effect of toxin on intestinal myoelectric activity (Mathias *et al.*, 1980).

7. THERAPY

7.1. The Problem of Antimicrobial Resistance

A number of studies over the years have demonstrated the efficacy of antimicrobials in treatment of shigellosis in shortening both the course of clinical disease and convalescent excretion of the organism when an absorbable drug is chosen and the isolate is sensitive to the agent employed. Over the past 25 years,

however, the problem of satisfying the latter criterion has become difficult. During the early 1950s, increasing resistance to sulfonamide was found in Japan. Indeed, over the decade from 1945 to 1955, the uniformly sensitive shigellas became just as uniformly resistant. As newer drugs were introduced, initial success was often followed by therapeutic failure as resistance to the new agents also emerged. In the latter half of the decade, organisms resistant to two or more antimicrobials also appeared. The observation that administration of a single drug to a patient infected with a sensitive strain could result in the sudden emergence of multiply antibiotic-resistant clones suggested to Akiba *et al.* (1960) and Ochiai *et al.* (1959) and their co-workers that resistance might be transferred to the pathogen from other bacteria present in the gastrointestinal tract (Farrar *et al.*, 1972).

In vitro studies quickly demonstrated this to be true and that the nature of the transferable factor was an extrachromosomal circular DNA called a plasmid (Watanabe, 1963). It is now known that transferable plasmids carry genetic information for many traits other than antimicrobial resistance, including heavy metal resistance and virulence factors such as toxins or bacterial surface structures (Smith and Linggood, 1971). Antimicrobial resistance plasmids, termed R factors, can also contain short segments of DNA known as transposons, which are flanked by inverted repeats of the purine and pyrimidine bases, possessing the ability to jump from plasmid to plasmid or to insert themselves into the bacterial chromosome (So *et al.*, 1979). Some transposons carry antibiotic resistance (e.g., ampicillin), whereas others may mediate other properties of virulence (Olarie *et al.*, 1976; So *et al.*, 1979).

The result of these mechanisms to move genetic information with survival value (e.g., antibiotic resistance) from strain to strain and species to species under selective pressure of antibiotic usage has been the widespread dissemination of antimicrobial resistance in *Shigella* species around the world (Table II). Plasmid biology is complex; successful transfer is determined by the nature of the plasmid itself (including whether it is compatible with other plasmids already present in the organism), whether or not it is mobilizable or requires additional factors to accomplish this, and on properties of the host bacterium that determine whether or not transfer will be stable. The genus *Shigella* seems to be adept at acquiring and retaining multiple R factors and antibiotic resistance transposons (Mata *et al.*, 1970; Olarie *et al.*, 1976; Rahaman *et al.*, 1975b).

Some investigators have raised the possibility that certain antibiotic R factors may be associated with the ability of the organism to disseminate in nature and to cause mini or maxi (epidemic) outbreaks (Gangarosa *et al.*, 1972). The recent epidemics of *S. dysenteriae* 1 infection in Mexico and Central America and in Bangladesh were caused by strains bearing a plasmid for resistance to tetracycline, chloramphenicol, streptomycin, sulfonamide, and, in addition in some, the ampicillin resistance transposon, TnA (Mata *et al.*, 1970; Olarie *et al.*,

Table II
Antibiotic Resistance of Shigellas Isolated in Different Countries

Country	Reference	<i>Shigella</i> species	Percent multiply resistant	Common resistance pattern ^a	Transfer demonstrated
Australia	Thomas <i>et al.</i> (1974)	<i>sonnei</i>	73% (27/37)	SM/Su	NT ^b
Bangladesh	Rahaman <i>et al.</i> (1974)	<i>dysenteriae</i> 1	5 strains	TC/CM/SM/Su/AM	Yes
India	Macaden <i>et al.</i> (1980)	<i>dysenteriae</i> 1	93% (134/144)	TC/CM/SM/AM	NT
	Paniker <i>et al.</i> (1978)	<i>dysenteriae</i> 1	66% (45/68)	TC/CM/SM/Su/AM	Yes
		<i>flexneri</i>	25% (6/24)	TC/CM/SM/Su/Am	Yes
Iran	Badalian and Tavakoli (1976)	<i>sonnei</i>	52% (89/172)	TC/CM/SM/Su	Yes
Malaysia	Sng and Lam (1971)	<i>flexneri</i>	12% (3/26)	TC/CM/SM/Su	Yes
		<i>sonnei</i>	11% (2/18)	TC/CM/SM/Su	Yes
Mexico	Olarte <i>et al.</i> (1976)	<i>dysenteriae</i> 1	5 strains	TC/CM/SM/Su/AM	Yes
New Zealand	Bremner (1978)	<i>sonnei</i> >> <i>flexneri</i>	98% (42/45)	TC/SM/Su/AM/CE	Yes
				AM/CE	Yes
Poland	Tkaczowa <i>et al.</i> (1977)	<i>sonnei</i>	63% (1599/2538)		NT
Somalia	Mero (1976)	<i>dysenteriae</i> 1 >> <i>flexneri</i>	87% (207/240)	TC/CM/SM/Su/AM	NT
Sweden	Urban (1972)	<i>flexneri</i>	52% (16/31)	TC/CM/Su/AM	Yes
		<i>sonnei</i>	25% (16/63)	TC/SM/Su/AM	Yes
Taiwan	Hsu and Tsan (1972)	<i>flexneri</i> >> <i>sonnei</i>	83% (156/187)	TC/CM/SM/Su	NT
U.S.A., New York	Lerman <i>et al.</i> (1973)	<i>sonnei</i> >> <i>flexneri</i>	75% (103/138)	TC/SM/Su/Am	NT
U.S.A., Houston	Byers <i>et al.</i> (1976)	<i>flexneri</i> <i>sonnei</i>	61% (34/56) 63% (71/113)	TC/CM/SM TC/SM/AM	NT

^aTC, tetracycline; CM, chloramphenicol; SM, streptomycin; Su, sulfonamide; AM, ampicillin; CE, cephalothin.

^bNT, not tested.

1976; Rahaman *et al.*, 1975b). In this regard, the epidemic organism differed from the *S. dysenteriae* strain that was occasionally isolated from patients in Guatemala during preepidemic years (Gordon *et al.*, 1965). Shortly thereafter, large numbers of typhoid bacilli were isolated from patients in Mexico (and in some Asian countries), and this strain had acquired a similar, but not identical, plasmid as well as the TnA transposon in some (Datta and Olarte, 1974).

An argument can be raised against the involvement of the plasmid in epidemic dissemination (at least in Central America), for Shiga dysentery is not a continuing problem, even though new susceptibles are born every year, and shigellosis continues to be a problem. In addition, it should be pointed out that the antibiotic resistance markers of the plasmid may be entirely innocent in this context of epidemic potential, but rather other properties on the same (or different) plasmid could be responsible. Properties of a plasmid are defined only when there is a system available to detect function (such as drug or heavy metal susceptibility); plasmids with no apparent function, termed cryptic, are certainly well described, and a known plasmid could contain cryptic regions or transposons for other critical properties.

The overall picture is not entirely grim, however. The antimicrobial resistance pattern of *Shigella* in different ecosystems is not simply progressive accumulation of R factors. Loss of resistance for individual drugs can also be documented over time (Table III) (Meyer and Lerman, 1980), perhaps in part related to patterns of antimicrobial usage in the community, but clearly not entirely. For example, in data from Iran (Badalin and Mohadjer, 1972), it would

Table III
Changes in Antimicrobial Sensitivity of *Shigella* over Time
in Two Distant Ecosystems

	Percent resistant		
	1962-1964	1965-1966	1967-1968
Iran (Badalian and Mohadjer, 1972)			
Tetracycline	67	80	12
Chloramphenicol	65	40	39
Streptomycin	17	10	6
Sulfonamide	18	6	0
New York (Meyer and Lerman, 1980)	1968-1970	1973-1974	1977-1978
Tetracycline	18	61	76
Chloramphenicol	3	0	3
Streptomycin	12	64	10
Sulfonamide	5	40	50
Ampicillin	0	68	7
Multiple drugs	14	72	40

be hard to explain a drop in tetracycline resistance from 80% in 1965–1966 to 12% in 1967–1968 on the basis of changes in use of tetracycline. Similarly, and more recently, it would be equally unlikely that significant changes in ampicillin consumption in New York could account for the extreme drop in resistance from 68% in 1973–1974 to only 7% in 1977–1978 (Meyer and Lerman, 1980).

What these data do clearly tell us is that antimicrobial resistance patterns must be continuously monitored in each geographic locale in order to provide up-to-date guidelines for antibiotic choice in shigellosis. This is exemplified by the several recent reports of excellent results with trimethoprim–sulfamethoxazole combinations at the same time that others have found a significant incidence of *in vitro* resistance to locally obtained isolates (Barada and Guerrant, 1980; Chang *et al.*, 1977; Mero, 1976; Nelson *et al.*, 1976a,b; Thomas *et al.*, 1974).

7.2. General Principles of Antimicrobial Choice

With this as a background, what are the general principles of antimicrobial usage in shigellosis? The most systematic studies of therapy for *Shigella* infections have been conducted by Nelson and Haltalin and their colleagues (Haltalin, 1973; Haltalin and Nelson, 1972; Haltalin *et al.*, 1967, 1968, 1972, 1973; Nelson and Haltalin, 1974). Three important principles of therapy emerge from these studies. First, the organism must be susceptible *in vitro*. This is, of course, one of the most fundamental principles in choice of antimicrobial for all infectious diseases. However, the converse, that an organism susceptible *in vitro* will necessarily respond *in vivo*, is not true.

In *Shigella* infection, the latter leads directly to the second principle for antimicrobial choice, that the agent employed must be systemically absorbed. Nonabsorbable antimicrobials do not have a significant therapeutic effect, presumably because they do not reach the invasive tissue microbial population. A third principle, that the agent should not be too well absorbed, comes from observations of the efficacy of amoxicillin therapy (Nelson and Haltalin, 1974). This drug, like ampicillin, satisfies the first two criteria and differs only in the high degree of systemic absorption compared to ampicillin, yet it does not work well. The logical explanation is a requirement for both tissue and luminal levels of effective drug.

7.3. Drugs of Choice

With these principles in mind, therapeutic choices can be suggested. When the organism is susceptible, ampicillin is an excellent drug to use because of efficacy, availability, and safety in both adults and children. Tetracycline, and perhaps particularly an agent like doxycycline which has an enterohepatic circulation and thus reaches both tissue and gut contents in high concentration, is an

acceptable choice for adults and children over the age of 10 (Keusch, 1979). It should not be used for younger children because of the deposition of tetracyclines in bone and growing teeth and the permanent damage that may ensue. Pickering *et al.* (1978) have recently reported success in treating both tetracycline-sensitive and -resistant *Shigella* strains with a bolus dose of 2.5 g of tetracycline. Apparently the concentrations achieved *in vivo* are sufficient to overcome the block of antibiotic uptake by resistant strains, which is the principle mechanism of R-factor-mediated tetracycline resistance rather than enzymatic inactivation of the drug as is the case for most other drugs.

A good alternative to ampicillin (and, in my opinion, the drug of next choice) for resistant strains or for patients allergic to the penicillins is the fixed-dose combination of trimethoprim and sulfamethoxazole (Barada and Guerrant, 1980; Chang *et al.*, 1977; Nelson *et al.*, 1976a,b). However, resistant strains have been isolated, and these might present a therapeutic dilemma (Mero, 1976; Thomas *et al.*, 1974). In this situation, naladixic acid (55 mg/kg per day for 5 days) can be used, although results are inferior to either ampicillin or trimethoprim-sulfamethoxazole (Haltalin *et al.*, 1973). It should be noted that this is a nonapproved use of naladixic acid in the United States and would necessitate informed consent of the patient at the very least.

Finally, it might be mentioned that trimethoprim-sulfamethoxazole has been successfully used to eradicate the chronic carrier state in both humans and monkeys (Chang *et al.*, 1977; Pucak *et al.*, 1977).

7.4. Nonspecific Therapy

Nonspecific therapy of shigellosis can be considered under three categories: fluid replacement, use of motility inhibitors, and treatment of febrile seizures (Keusch, 1979).

Therapy of fluid and electrolyte losses is, in principle, no different from the therapy of other dehydrating diarrheal diseases. Oral fluid replacement with glucose-electrolyte solutions such as the formulation recommended by the World Health Organization may be employed. Although sugar transport abnormalities have been found in the ileum of experimental animals exposed to *Shigella* toxin (Binder and Whiting, 1977), the glucose-facilitated cotransport of sodium occurs primarily in proximal intestine, and this is probably intact in shigellosis. Because massive choleralike dehydration is unusual in shigellosis, oral therapy should suffice in most individuals without the need to employ intravenous rehydration.

The use of motility inhibitors such as diphenoxylate is considered by some authorities (DuPont and Hornick, 1973) to be contraindicated in shigellosis for two reasons. First, the resulting intestinal stasis may permit further invasion of epithelial cells by shigellas as a consequence of prolonging the contact of organ-

isms with the mucosa. In this view, hypermotility is teleologically seen as a host response to rid himself of the offending pathogen, and any measure that precludes this defensive response can worsen the severity of infection. A second reason to avoid these agents is that they, like the infection itself, may lead to toxic dilatation of the colon, which is a serious and often fatal complication (Mata and Castro, 1974; Smith *et al.*, 1962). It would seem unwise to add one potential cause of toxic dilatation to another already present. The wisest course at this time is to treat shigellosis specifically with appropriate antimicrobials and in this way to improve the symptoms of hypermotility.

Febrile convulsions in shigellosis are as much a medical emergency as febrile convulsions from any other cause. They generally occur in the young infant or child and must be treated (Keusch, 1979). In addition to insuring the patency of the airway, preventing laceration of the tongue, and preventing aspiration, phenobarbital (10 mg/kg) can be given as an intramuscular loading dose or intravenous diazepam (up to 0.2 mg/kg over 5 min or until the seizure ceases) can be employed. Steps should be taken to reduce body temperature by promoting heat loss across the skin and use of small doses of aspirin, according to age and size of the patient, on a regular 4-hr schedule to prevent further rapid and excessive rises in temperature.

8. SUMMARY

Shigellosis is an acute infection of the gastrointestinal tract, affecting the proximal small bowel and large bowel, and resulting in both diarrhea and dysentery. Clinical manifestations may range from mild to severe, particularly in young children with marginal nutritional status. In these latter subjects, the infection may disseminate to the bloodstream and terminate fatally, whereas it is generally self-limited in the otherwise healthy.

Few bacteria are needed to produce clinical illness. As a consequence, infection spreads by person-to-person contact. Secondary attack rates are high, particularly among the more susceptible childhood populations. Both food-borne and water-borne transmission may occur as well, so that the incidence rates in preschool children in the developing countries approximate 200,000 cases per 100,000 population per year. Clinical manifestations are more severe in the poorly nourished child, and the disease is prolonged, accounting for the important adverse effect on growth, development, and nutrition of *Shigella* infection in such children.

Diagnosis is relatively simple, even with relatively simple microbiological laboratory facilities; however, care must be given to the collection of appropriate specimens and handling and shipping to the laboratory. Specific antimicrobial treatment is useful, but it may be difficult to select a drug to which the organism

is susceptible because of widespread R-factor-mediated resistance. Fluid and electrolyte therapy can be readily administered orally using a glucose–electrolyte formula, and severe dehydration is unusual. Finally, febrile convulsions may be seen in predisposed subjects, a medical emergency that requires prompt medical attention.

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REFERENCES

- Akiba, T., Koyama, K., Ishiki, Y., Kimura, S., and Fukushima, J., 1960, On the mechanism of development of multiple drug-resistant clones of *Shigella*, *Jpn. J. Microbiol.* 4:219–227.
- Badalian, K., and Mohadjer, S., 1972, Antibiotic sensitivity changes in enteric pathogens isolated in central plateau of Iran (a nine year report), *J. Trop. Med. Hyg.* 75:45–52.
- Badalian, K., and Tavakoli, H., 1976, Transfer of drug resistance factor in *Shigella sonnei* isolated in Iran, *Pahlavi Med. J.* 7:71–91.
- Baine, W. B., Herron, C. A., Bridson, K., Barker, W. H., Jr., Lindell, S., Mallison, G. F., Wells, J. G., Martin, W. T., Kosuri, M. R., Carr, F., and Voelker, E., Sr., 1975, Waterborne shigellosis at a public school, *Am. J. Epidemiol.* 101:323–332.
- Barada, F. A., Jr., and Guerrant, R. L., 1980, Sulfamethoxazole–trimethoprim versus ampicillin in treatment of acute invasive diarrhea, *Antimicrob. Agents Chemother.* 17:961–964.
- Barrett-Connor, E., and Connor, J. D., 1969, Skin lesions and shigellosis, *Am. J. Trop. Med. Hyg.* 18:555–558.
- Barrett-Connor, E., and Connor, J. D., 1970, Extraintestinal manifestations of shigellosis, *Am. J. Gastroenterol.* 53:234–245.
- Binder, H. J., and Whiting, D. S., 1977, Inhibition of small-intestinal sugar and amino acid transport by the enterotoxin of *Shigella dysenteriae* 1, *Infect. Immun.* 16:510–512.
- Black, R. E., Graun, G. F., and Blake, P. E., 1978, Epidemiology of common-source outbreaks of shigellosis in the United States, 1961–1975, *Am. J. Epidemiol.* 108:47–52.
- Bremner, D. A., 1978, The transfer of antibiotic resistance by R plasmids in *Shigellae*, *N.Z. Med. J.* 88:9–10.
- Brown, J. E., Ussery, M. A., Leppla, S. H., and Rothman, S. W., 1980, Inhibition of protein synthesis by Shiga toxin: Activation of the toxin and inhibition of peptide elongation, *FEBS Lett.* 117:84–88.
- Byers, P. A., DuPont, H. L., and Goldschmidt, M. C., 1976, Antimicrobial susceptibilities of shigellae isolated in Houston, Texas in 1974, *Antimicrob. Agents Chemother.* 9:288–291.
- Cáceres, A., and Mata, L. J., 1974, Serologic response of patients with Shiga dysentery, *J. Infect. Dis.* 129:439–443.
- Calin, A., and Fries, J. F., 1976, An “experimental” epidemic of Reiter’s syndrome revisited. Follow-up evidence on genetic and environmental factors, *Ann. Intern. Med.* 84:564–566.
- Castro, F., Rosal, J. E., and Mata L. J., 1974, Hallazgos anatomopatológicos en 173 casos de disenteria bacilar, in: *Simposio Sobre Disentería Shiga en Centroamérica*, PAHO (WHO), Washington, pp. 17–26.
- Chang, M. J., Dunkle, L. M., VanRenken, D., Anderson, D., Wong, M. L., and Feigan, R. D.,

- 1977, Trimethoprim-sulfamethoxazole compared to ampicillin in the treatment of shigellosis, *Pediatrics* 59:726-729.
- Charney, A. N., Gots, R. E., Formal, S. B., and Giannella, R. A., 1976, Activation of intestinal mucosal adenylate cyclase by *Shigella dysenteriae* 1 enterotoxin, *Gastroenterology* 70:1085-1090.
- Chen, L. C., Chowdhury, A. K. M. A., and Huffman, S. L., Anthropometric assessment of energy-protein malnutrition and subsequent risk of mortality among preschool aged children, *Am. J. Clin. Nutr.* 33:1836-1845.
- Conradi, H., 1903, Ueber lösliche, durch aseptique Autolyse erhaltene Giftstoffe von Ruhr- und Typhus-bazillen, *Dtsch. Med. Wochenschr.* 29:26-28.
- Cooper, J. E., and Needham, J. R., 1976, An outbreak of shigellosis in laboratory marmosets and tamarins (Family: Callithricidae), *J. Hyg.* 76:415-424.
- Corwin, L. M., Rothman, S. W., Kim, R., and Talevi, L. A., 1971, Mechanisms and genetics of resistance to sodium lauryl sulfate in strains of *Shigella* and *Escherichia coli*, *Infect. Immun.* 4:287-294.
- Corwin, L. M., and Talevi, L. A., 1972, Mutation in *Shigella flexneri* 2a resulting in an increased electrophoretic mobility, *Infect. Immun.* 5:798-802.
- Dale, D. C., and Mata, L. J., 1968, Studies of diarrheal disease in Central America. XI. Intestinal bacterial flora in malnourished children with shigellosis, *Am. J. Trop. Med.* 17:397-403.
- Datta, N., and Olarte, J., 1974, R factors in strains of *Salmonella typhi* and *Shigella dysenteriae* 1 isolated during epidemics in Mexico: Classification by compatibility, *Antimicrob. Agents Chemother.* 5:310-317.
- Donadio, J., and Gangarosa, E., 1969, Foodborne shigellosis, *J. Infect. Dis.* 119:666-668.
- Drachman, R. H., Payne, F. J., Jenkins, A. A., Mackel, D. C., Petersen, N. J., Boring, J. R. III, Gareau, F. E., Fraser, R. S., and Myers, G. G., 1960, An outbreak of water borne *Shigella* gastroenteritis, *Am. J. Hyg.* 72:321-334.
- DuPont, H. L., and Hornick, R. B., 1973, Adverse effect of Lomotil therapy in shigellosis, *J.A.M.A.* 226:1525-1528.
- DuPont, H. L., Hornick, R. B., Dawkins, A. T., Snyder, M. J., and Formal, S. B., 1969, The response of man to virulent *Shigella flexneri* 2a, *J. Infect. Dis.* 119:296-299.
- DuPont, H. L., Gangarosa, E. J., Reller, L. B., Woodward, W. E., Armstrong, R. W., Hammond, J., Glaser, K., and Morris, G. K., 1970, Shigellosis in custodial institutions, *Am. J. Epidemiol.* 92:172-179.
- DuPont, H. L., Formal, S. B., Hornick, R. B., Snyder, M. J., Libonati, J. P., Sheahan, D. G., LaBrec, E. H., and Kalas, J. P., 1971, Pathogenesis of *Escherichia coli* diarrhea, *N. Engl. J. Med.* 285:1-9.
- DuPont, H. L., Hornick, R. B., Snyder, M. J., Libonati, J. P., Formal, S. B., and Gangarosa, E. J., 1972, Immunity in shigellosis. I. Response of man to attenuated shigella strains, *J. Infect. Dis.* 125:5-11.
- Ewing, W. H., and Martin, W. J., 1974, Enterobacteriaceae, in: *Manual of Clinical Microbiology* (E. H. Lennett, E. H., Spaulding, J. P. Truant, eds.), 2nd ed, American Society Microbiology, Washington, pp. 189-221.
- Farrar, W. E., Jr., Eidson, M., Guerry, P., Falkow, S., Drusin, L. M., and Roberts, R. B., 1972, Interbacterial transfer of R-factor in the human intestine: *In vivo* acquisition of R-factor mediated kanamycin resistance by a multi-resistant strain of *Shigella sonnei*, *J. Infect. Dis.* 126:27-33.
- Feldman, R. A., Bhat, P., and Kamath, K. R., 1970, Infection and disease in a group of South Indian families. IV. Bacteriologic methods and a report of the frequency of enteric bacterial infection in preschool children, *Am. J. Epidemiol.* 92:367-375.
- Flexner, S., 1900, On the etiology of tropical dysentery, *Bull. Johns Hopkins Hosp.* 11:231-242.

- Flores, J., Grady, G. F., McIver, J., Witkum, P., Beckman, B., and Sharp, G. W. G., 1974, Comparison of the effects of enterotoxins of *Shigella dysenteriae* and *Vibrio cholerae* on the adenylate cyclase system of the rabbit ileum, *J. Infect. Dis.* 130:374–379.
- Floyd, T. M., Higgins, A. R., and Kadar, M. A., 1956, Studies in shigellosis: V. The relationship of age to the incidence of *Shigella* infections in Egyptian children, with special reference to shigellosis in the newborn and in infants in the first six months of life, *Am. J. Trop. Med.* 5:119–130.
- Formal, S. B., LaBrec, E. H., and Schneider, H., 1965a, Pathogenesis of bacillary dysentery in laboratory animals, *Fed. Proc.* 24:29–34.
- Formal, S. B., LaBrec, E. H., Kent, T. H., and Falkow, S., 1965b, Abortive intestinal infection with an *Escherichia coli*-*Shigella flexneri* hybrid strain, *J. Bacteriol.* 89:1374–1382.
- Formal, S. B., Kent, T. H., May, H. C., Palmer, A., Falkow, S., and LaBrec, E. H., 1966, Protection of monkeys against experimental shigellosis with a living attenuated oral polyvalent dysentery vaccine, *J. Bacteriol.* 92:17–22.
- Formal, S. B., LaBrec, E. H., Hornick, R. B., DuPont, H. L., and Snyder, M. J., 1971, Attenuation of strains of dysentery bacilli, in: *International Symposium on Enterobacterial Vaccines* (R. H. Reganey, M. Stanic, J. Ungar, eds.), Volume 15, Karger, Basel, pp. 73–79.
- Fox, J. G., 1975, Transmissible drug resistance in *Shigella* and *Salmonella* isolated from pet monkeys and their owners, *J. Med. Primatol.* 4:165–171.
- Gangarosa, E. J., Perera, D. R., Mata, L. J., Mendizábol-Morris, C., Guzmán, G., and Reller, L. B., 1970, Epidemic Shiga bacillus dysentery in Central America. II. Epidemiologic studies in 1969, *J. Infect. Dis.* 122:181–190.
- Gangarosa, E. J., Bennett, J. V., Wyatt, C., Pierce, P. E., Olarte, J., Hernandez, P. M., Vasquez, V., and Bessudo, M. D., 1972, An epidemic associated episome? *J. Infect. Dis.* 126:215–218.
- Gemski, P., Jr., Takeuchi, A., Washington, O., and Formal, S. B., 1972a, Shigellosis due to *Shigella dysenteriae* 1: Relative importance of mucosal invasion vs. toxin production in pathogenesis, *J. Infect. Dis.* 126:523–530.
- Gemski, P., Jr., Sheahan, D. G., Washington, O., and Formal, S. B., 1972b, Virulence of *Shigella flexneri* hybrids expressing *Escherichia coli* somatic antigens, *Infect. Immun.* 6:104–111.
- Gibbons, R. A., Sellwood, R., Burrows, M., and Hunter, P. A., 1977, Inheritance of resistance to neonatal *E. coli* diarrhoea in the pig: Examination of the genetic system, *Theor. Appl. Genet.* 51:65–70.
- Gordon, J. E., Ascoli, W., Pierce, V., Guzmán, M. A., and Mata, L. J., 1965, Studies of diarrheal disease in Central America. VI. An epidemic of diarrhea in a Guatemalan highland village, with a component due to *Shigella dysenteriae* type 1, *Am. J. Trop. Med. Hyg.* 14:404–411.
- Gots, R. E., Formal, S. B., and Giannella, R. A., 1974, Indomethacin inhibition of *Salmonella typhimurium*, *Shigella flexneri* and cholera mediated rabbit ileal secretion, *J. Infect. Dis.* 130:280–284.
- Grady, G. F., and Keusch, G. T., 1971, Pathogenesis of bacterial diarrheas, *N. Engl. J. Med.* 285:831–841, 891–900.
- Hale, T. L., and Formal, S. B., 1981, Protein synthesis in HeLa or Henle 407 cells infected with *Shigella dysenteriae* 1, *S. flexneri* 2a or *Salmonella typhimurium* W118, *Infect. Immun.* 32:137–144.
- Haltalin, K. C., 1967, Neonatal shigellosis. Report of 16 cases and review of the literature, *Am. J. Dis. Child.* 114:603–611.
- Haltalin, K. C., 1973, Ampicillin and shigellosis, *Am. J. Dis. Child.* 125:458.
- Haltalin, K. C., and Nelson, J. D., 1965, Coliform septicemia complicating shigellosis in children, *J.A.M.A.* 192:441–443.
- Haltalin, K. C., and Nelson, J. D., 1972, Failure of furazolidone therapy in shigellosis, *Am. J. Dis. Child.* 123:40–44.

- Haltalin, K. C., Nelson, J. D., Ring, R. III, Sladoje, M., and Hinton, L. V., 1967, Double-blind treatment study of shigellosis comparing ampicillin sulfadiazine and placebo, *J. Pediatr.* 70:970-981.
- Haltalin, K. C., Nelson, J. D., Hinton, L. V., Kusmiesz, H. T., and Sladoje, M., 1968, Comparison of orally absorbable and non-absorbable antibiotics in shigellosis, *J. Pediatr.* 72:708-720.
- Haltalin, K. C., Kusmiesz, H. T., Hinton, L. V., and Nelson, J. D., 1972, Treatment of acute diarrhea in outpatients, *Am. J. Dis. Child.* 124:554-561.
- Haltalin, K. C., Nelson, J. D., and Kusmiesz, H. T., 1973, Comparative efficacy of nalidixic acid and ampicillin for severe shigellosis, *Arch. Dis. Child.* 48:305-312.
- Hardy, A. V., and Watt, J., 1945, Studies of the acute diarrheal diseases XIV. Clinical observations, *Pub. Health Rep.* 60:521-532.
- Harris, J. C., DuPont, H. L., and Hornick, R. B., 1972, Fecal leukocytes in diarrheal illness, *Ann. Intern. Med.* 76:697-703.
- Hsu, W. H., and Tsan, Y. C., 1972, Drug resistance pattern of *Shigella* isolated in Taiwan during 1970-1971, *J. Formosan Med. Assoc.* 71:530-536.
- Jao, R. L., and Jackson, G. G., 1963, Asymptomatic urinary-tract infection with *Shigella sonnei* in a chronic fecal carrier, *N. Engl. J. Med.* 268-1165-1168.
- Keusch, G. T., 1979, *Shigella* infections, *Clin. Gastroenterol.* 8:645-662.
- Keusch, G. T., 1981, Receptor mediated endocytosis of shigella cytotoxin, in: *Receptor Mediated Binding and Internalization of Toxins and Hormones*, (J. Middlebrook and L. Kohn, eds.), Academic Press, New York, pp. 95-110.
- Keusch, G. T., 1982, Shigellosis, in: *Bacterial Infections of Humans*, (A. S. Evans and H. Feldman, eds.), Plenum Press, New York, in press.
- Keusch, G. T., and Jacewicz, M., 1973, Serum enterotoxin neutralizing antibody in human shigellosis, *Nature [New Biol.]* 241:31-32.
- Keusch, G. T., and Jacewicz, M., 1975, The pathogenesis of shigella diarrhea. V. Relationship of Shiga enterotoxin, neurotoxin and cytotoxin, *J. Infect. Dis.* 131:S33-S39.
- Keusch, G. T., and Jacewicz, M., 1977a, The pathogenesis of shigella diarrhea. VI. Toxin and antitoxin in *Shigella flexneri* and *Shigella sonnei* infections in humans, *J. Infect. Dis.* 135:552-556.
- Keusch, G. T., and Jacewicz, M., 1977b, Pathogenesis of shigella diarrhea. VII. Evidence for a cell membrane toxin receptor involving $\beta 1 \rightarrow 4$ linked N-acetyl-D-glucosamine oligomers, *J. Exp. Med.* 146:535-546.
- Keusch, G. T., Grady, G. F., Mata, L. J., and McIver, J., 1972a, The pathogenesis of *Shigella* diarrhea. I. Enterotoxin production by *Shigella dysenteriae* 1, *J. Clin. Invest.* 51:1212-1218.
- Keusch, G. T., Grady, G. F., Takeuchi, A., and Sprinz, H., 1972b, The pathogenesis of *Shigella* diarrhea. II. Enterotoxin induced acute enteritis in the rabbit ileum, *J. Infect. Dis.* 126:92-95.
- Keusch, G. T., Jacewicz, M., Levine, M. M., Hornick, R. B., and Kochwa, S., 1976, Pathogenesis of *Shigella* diarrhea. Serum anticytotoxin antibody response produced by toxigenic and non-toxigenic *Shigella dysenteriae* 1, *J. Clin. Invest.* 57:194-202.
- Keusch, G. T., Donohue-Rolfe, A., and Jacewicz, M., 1982, *Shigella* toxin(s): Description and role in diarrhea and dysentery, *Pharmacol. Ther.*, in press.
- Kim, R., and Corwin, L. M., 1974, Mutation in *Shigella flexneri* resulting in loss of ability to penetrate HeLa cells and loss of glycerol kinase activity, *Infect. Immun.* 9:916-923.
- Kopecko, D. J., Washington, O., and Formal, S. B., 1980, Genetic and physical evidence for plasmid control of *Shigella sonnei* Form I cell surface antigen, *Infect. Immun.* 29:207-214.
- Korzeniowski, O. M., Barada, F. A., Rouse, J. D., and Guerrant, R. L., 1979, Value of examination for fecal leukocytes in the early diagnosis of shigellosis, *Am. J. Trop. Med. Hyg.* 28:1031-1035.
- Koshi, G., Daniel, J., and Pereira, S. M., 1979, Septicaemic manifestations of shigellosis, *Indian J. Med. Res.* 70:916-922.

- Koster, F., Levin, J., Walker, L., Tung, K. S. K., Gilman, R. H., Rahaman, M. M., Majid, M. A., Islam, S., and Williams, R. C., Jr., 1978, Hemolytic-uremic syndrome after shigellosis. Relation to endotoxemia and circulating immune complexes, *N. Engl. J. Med.* 298:927-933.
- LaBrec, E. H., Schneider, H., Magnani, T. J., and Formal, S. B., 1964, Epithelial cell penetration is an essential step in the pathogenesis of bacillary dysentery, *J. Bacteriol.* 88:1503-1518.
- Lerman, S. J., Waller, J. M., and Simms, D. H., 1973, Resistance of *Shigellae* to ampicillin and other antibiotics: South Bronx, N.Y. (1971 and 1972), *J. Pediatr.* 83:500-501.
- Levine, M. M., DuPont, H. L., Formal, S. B., Hornick, R. B., Takeuchi, A., Gangarosa, E. J., Snyder, M. J., and Libonati, J. P., 1973, Pathogenesis of *Shigella dysenteriae* 1 (Shiga) dysentery, *J. Infect. Dis.* 127:261-270.
- Macaden, R., Gokul, B. N., Pereira, P., and Bhat, P., 1980, Bacillary dysentery due to multidrug resistant *Sh. dysenteriae* type 1, *Indian J. Med. Res.* 71:175-177.
- Mata, L. J., 1978, *The children of Santa Maria Cauque: A Prospective Field Study of Health and Growth*, The M.I.T. Press, Cambridge.
- Mata, L. J., and Castro, F., 1974, Epidemiology, diagnosis and impact of Shiga dysentery in Central America, *Industry Trop. Health* 8:30-37.
- Mata, L. J., Catalán, M. A., and Gordon, J. E., 1966, Studies of diarrheal disease in Central America. IX. *Shigella* carriers among young children of a heavily seeded Guatemalan convalescent home, *Am. J. Trop. Med. Hyg.* 15:632-638.
- Mata, L. J., Gangarosa, E. J., Cáceres, A., Perera, D. R., and Mejicanos, M. L., 1970, Epidemic Shiga bacillus dysentery in Central America. I. Etiologic investigations in Guatemala, 1969, *J. Infect. Dis.* 122:170-180.
- Mathias, J. R., Carlson, G. M., Martin, J. L., Shields, R. P., and Formal, S. B., 1980, *Shigella dysenteriae* 1. enterotoxin: Proposed role in pathogenesis of shigellosis. *Am. J. Physiol.* 239:G382-386.
- Mero, E., 1976, Resistance to antibiotics of *Shigella* strains isolated in Somalia, *Bull. WHO* 54:473-474.
- Merson, M. H., Tenney, J. H., Meyers, J. D., Wood, B. T., Wells, J. G., Rimzo, W., Cline, B., DeWitt, W. E., Skaliy, P., and Mallison, G. F., 1975, Shigellosis at sea. An outbreak aboard a passenger cruise ship, *Am. J. Epidemiol.* 101:165-175.
- Meyer, P. W., and Lerman, S. J., 1980, Rise and fall of *Shigella* antibiotic resistance, *Antimicrob. Agents Chemother.* 17:101-102.
- Moore, D. G., Yancey, R. J., Lankford, C. E., and Earhart, C. F., 1980, Bacteriostatic enterochelin-specific immunoglobulin from normal human serum, *Infect. Immun.* 27:418-423.
- Murphy, T. V., 1979, *Shigella* vaginitis: Report of 38 patients and review of the literature, *Pediatrics* 63:511-516.
- Music, S. I., Libonati, J. P., Wenzel, R. P., Synder, R. B., Hornick, R. B., and Woodward, T. E., 1970, Induced human cholera, in: *Antimicrobial Agents and Chemotherapy*. (G. R. Hobby, ed.), American Society for Microbiology, Washington, pp. 462-466.
- Neglia, T. G., Marr, T. J., and Davis, A. T., 1976, *Shigella* dysentery with secondary *Klebsiella sepsis*, *J. Pediatr.* 89:253-254.
- Nelson, J. D., and Haltalin, K. C., 1974, Amoxycillin less effective than ampicillin against *Shigella in vitro* and *in vivo*. Relationship of efficacy to activity in serum, *J. Infect. Dis.* 129:S222-S227.
- Nelson, J. D., Ksrmiesz, H., Jackson, L. H., and Woodman, E., 1976a, Trimethoprim-sulfamethoxazole therapy for shigellosis, *J.A.M.A.* 235:1239-1243.
- Nelson, J. D., Ksrmiesz, H., and Jackson, L. H., 1976b, Comparison of trimethoprim-sulfamethoxazole and ampicillin therapy for shigellosis in ambulatory patients, *J. Pediatr.* 89:491-493.
- Noer, H. R., 1966, An "experimental" epidemic of Reiters syndrome, *J.A.M.A.* 198:693-698.
- Ochiai, K., Yamanaka, T., Kimura, K., and Sowada, O., 1959, Studies on the inheritance of drug

- resistance between *Shigella* strains and *Escherichia coli* strains, *Nihon Iji Shimpō* 1861:34–46, 1959.
- Olarte, J., Filloy, L., and Galindo, E., 1976, Resistance of *Shigella dysenteriae* type 1 to ampicillin and other antimicrobial agents: Strains isolated during a dysentery outbreak in a hospital in Mexico City, *J. Infect. Dis.* 133:572–575.
- Olenick, J. G., and Wolfe, A. D., 1980, *Shigella* toxin inhibition of binding and translocation of polyuridylic acid by *Escherichia coli* ribosomes, *J. Bacteriol.* 141:1246–1250.
- Olsnes, S., and Eiklid, K., 1980, Isolation and characteristics of *Shigella shigae* cytotoxin, *J. Biol. Chem.* 225:284–289.
- Paniker, O. K., Vimala, K. N., Bhat, P., and Stephen, S., 1978, Drug resistant shigellosis in South India, *Indian J. Med. Res.* 68:413–417.
- Pickering, L. K., DuPont, H. L., Olarte, J., Conklin, R., and Ericsson, C., 1977, Fecal leukocytes in enteric infections, *Am. J. Clin. Pathol.* 68:562–565.
- Pickering, L. K., DuPont, H. L., Olarte, J., Conklin, R., and Ericsson, C., 1978, Single dose tetracycline therapy for shigellosis in adults, *J.A.M.A.* 239:853–854.
- Pucak, G. J., Orcutt, R. P., Judge, R. J., and Rendon, F., 1977, Elimination of the *Shigella* carrier state in rhesus monkeys (*Macaca malatta*) by trimethoprim–sulfamethoxazole, *J. Med. Primatol.* 6:127–132.
- Rahaman, M. M., Hud, I., Dey, C. R., Kibriya, A. K., and Curlin, G., 1974, Ampicillin resistant Shiga bacillus in Bangladesh, *Lancet* 1:406–407.
- Rahaman, M. M., Hug, I., and Dey, C. R., 1975a, Superiority of MacConkey's agar over *Salmonella-Shigella* agar for isolation of *Shigella dysenteriae* Type 1, *J. Infect. Dis.* 131:700–703.
- Rahaman, M. M., Alam, A. K. M. J., Islam, M. R., Greenough, W. B., and Lindenbaum, J., 1975b, Shiga bacillus dysentery associated with marked leukocytosis and erythrocyte fragmentation, *Johns Hopkins Med. J.* 136:65–70.
- Rahaman, M. M., Khan, M. M., Asiz, K. M. A., Islam, M. S., and Golam Kibriya, A. K. M., 1975c, An outbreak of dysentery caused by *Shigella dysenteriae* Type 1 on a coral island in the Bay of Bengal, *J. Infect. Dis.* 132:15–19.
- Reed, W. P., and Albright, E. L., 1974, Serum factors responsible for killing of *Shigella*, *Immunology* 26:205–215.
- Reisbig, R., Olsnes, S., and Eiklid, K., 1981, The cytotoxic activity of *Shigella* toxin. Evidence for catalytic inactivation of the 60S ribosomal subunit, *J. Biol. Chem.* 256:8739–8744.
- Roper, D. L., 1979, Corneal ulceration secondary to intestinal *Shigella sonnei*, *Arch. Ophthalmol.* 97:888–889.
- Rosenberg, M. L., Weissman, J. B., Gangarosa, E. J., Reller, L. B., and Beasley, R. P., 1976, Shigellosis in the United States: Ten year review of nationwide surveillance, *Am. J. Epidemiol.* 104:543–551.
- Rout, W. R., Formal, S. B., Giannella, R. A., and Dammin, G. J., 1975, Pathophysiology of *Shigella* diarrhea in the rhesus monkey: Intestinal transport, morphological and bacteriological studies, *Gastroenterology* 68:270–278.
- Satterwhite, T. K., Evans, D. G., DuPont, H. L., and Evans, D. J., Jr., 1978, Role of *Escherichia coli* colonization factor antigen in acute diarrhoea, *Lancet* 2:181–184.
- Scragg, J. N., Rubidge, C. J., and Appelbaum, P. C., 1978, *Shigella* infection in African and Indian children with special reference to *Shigella* septicemia, *J. Pediatr.* 93:796–799.
- Sellwood, R., Gibbons, R. A., Jones, G. W., and Rutter, J. M., 1975, Adhesion of enteropathogenic *Escherichia coli* to pig intestinal brush borders: The existence of two pig phenotypes, *J. Med. Microbiol.* 8:405–411.
- Shiga, K., 1898, Ueber den Dysenteriebacillus (*Bacillus dysenteriae*), *Zentralbl. Bakteriol.* [Orig. A] 24:913–918.
- Smith, F. W., Law, D. H., Nickel, W. F. Jr., and Slesinger, M. H., 1962, Fulminant ulcerative

- colitis with toxic dilatation of the colon: Medical and surgical management of 11 cases with observations regarding etiology, *Gastroenterology* 42:233–243.
- Smith, H. W., and Linggood, M. A., 1971, Observations on the pathogenic properties of the K88, HLY and ENT plasmids of *Escherichia coli* with particular reference to porcine diarrhoea, *J. Med. Microbiol.* 4:467–485.
- Sng, E. H., and Lam, S., 1971, Multiple drug resistance and the presence of R factors in enteric pathogens, *Med. J. Malaysia* 4:301–304.
- So., M., Heffron, F., and McCarthy, B. J., 1979, The *E. coli* gene encoding heat stable toxin is a bacterial transposon flanked by inverted repeats of IS1, *Nature* 277:453–456.
- Steinberg, S. E., Banwell, J. G., Yardley, J. H., Keusch, G. T., and Hendrix, T. R., 1975, Comparison of secretory and histological effects of shigella and cholera enterotoxins in rabbit jejunum, *Gastroenterology* 68:309–317.
- Thomas, E., Kostalas, G., and Beare, J. H., 1974, Antibiotic susceptibility of *Shigella* in Australia, *Lancet* 1:936.
- Thomas, M. E. M., and Tillett, H. E., 1973, Dysentery in general practice: A study of cases and their contacts in Enfield and an epidemiological comparison with salmonellosis, *J. Hyg. (Camb.)* 71:373–389.
- Thomas, M. E., Haiden, Y., and Datta, N., 1972, An epidemiological study of strains of *Shigella sonnei* from two related outbreaks, *J. Hyg. (Camb.)* 70:589–596.
- Tkaczowa, A., Durlakowa, I., and Weber, B., 1977, Investigations on drug-resistance of *Shigella sonnei* bacilli isolated in Poland in years 1959–1972, *Arch. Immunol. Ther. Exp.* 25:263–272.
- Turnbull, P. C. B., 1979, Food poisoning with special reference to salmonella—its epidemiology, pathogenesis and control, *Clin. Gastroenterol.* 8:663–714.
- Urban, T., 1972, Transferrable multiple drug resistance of *Shigella* strains isolated in Sweden, *Scand. J. Infect. Dis.* 4:221–227.
- Watanabe, T., 1963, Infective heredity of multiple drug resistance in bacteria, *Bacteriol. Rev.* 27:87–115.
- Weissman, J. B., Schmerler, A., Weiler, P., Filice, G., Godbey, N., and Hansen, I., 1974, The role of preschool children and day-care centers in the spread of shigellosis in the community, *J. Pediatr.* 84:797–802.
- Yoshikawa, M., 1972, Reduced plating efficiency on *Salmonella-Shigella* agar of strains of *Enterobacteriaceae* carrying transferable drug-resistance factors, *J. Infect. Dis.* 125:310–312.

Giardiasis

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1. INTRODUCTION

Giardiasis in humans is caused by a flagellated protozoan *Giardia lamblia* first described by Anton van Leeuwenhoek in 1681. The organism is a common cause of diarrhea, especially chronic and/or recurrent disease, in developing countries where generally both sanitation and personal hygiene are poor. With today's ease of air travel, however, giardiasis is being seen with increasing frequency in North America. About 4.1% of stool specimens submitted to a state public health laboratory in the United States were found to contain *Giardia lamblia*, indicating the magnitude of the problem (Center for Disease Control, 1978). About 35 years ago, the trophozoite of *Giardia lamblia* was considered a commensal of the gastrointestinal tract. On the basis of evidence obtained in a number of epidemiologic studies and experiments in animal models, it is clear that *Giardia* is pathogenic. *Giardia lamblia* is now regarded as the commonest intestinal parasite in North America, which, although not fatal, can cause a lot of misery, malabsorption, and debilitation. Giardiasis is relatively easy to treat. The physician should keep a high index of suspicion to diagnose this disorder; otherwise the disease might be missed and take a serious turn, necessitating referral to a regional hospital and consultation with a specialist.

2. HISTORICAL ASPECTS

Anton van Leeuwenhoek, while examining his own stools during about of long-standing diarrhea, first identified this organism in 1681 (Dobell, 1932).

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Vilem Dusan Fedorovich Lambl, a Czech, in 1859 noted this organism in humans and called it *Corcomonas intestinales*. Kunstler (1882) observed this species in tadpoles and was first to name it *Giardia*. Six years later, in 1888, Blanchard unsuccessfully tried to honor Lambl by trying to name the organism *Lamblia*. Alexeieff (1914) showed that human giardiasis is a member of the species observed by Kunstler (1882). Kofoed (1920) and Dobell (1940) studied the history of nomenclature of this organism and both came to similar conclusions that the organism should be called *Giardia* and not *Lamblia*; however, the name recommended by Charles Wardell Stiles, i.e., *Giardia lamblia*, remained in this confusion of nomenclature. Today, this protozoan is known as *Giardia* in western countries and as *Lamblia* in the Soviet Union and eastern Europe. More than 200 years have elapsed between the initial discovery of the parasite and the establishment of its relationship to disease.

3. PATHOGEN

Giardia lamblia exists in two forms, trophozoite stage and cyst stage (Figure 1). The trophozoite measures 5–9 by 10–12 μm . It is rounded anteriorly and pointed at its posterior end. The organism is flattened dorsoventrally and is

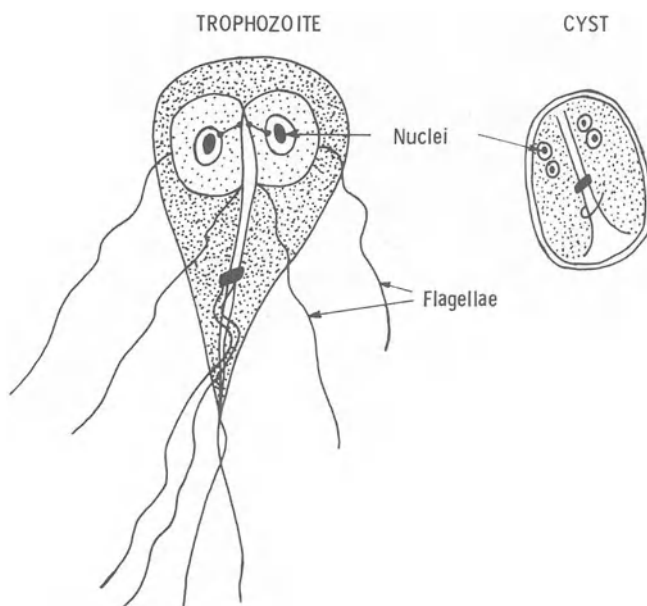


Figure 1. Morphology of *Giardia* trophozoite and cyst.

convex on the dorsal surface. The bilobed adhesive disk is on its concave ventral surface, and along with the ventral flagella, this structure helps the organism to get a firm grip on the intestinal mucosa of the host. There are a total of four pairs of flagella, anterior, posterior, ventral, and caudal, which arise from kinetosomes located between the anterior portion of the two nuclei. The median bodies are a pair of large curved bodies lying behind the ventral disk; their function is obscure, although it has been suggested that they may help to support the posterior end of the organism or may be involved in its energy metabolism. It is to be noted that there are no mitochondria, smooth endoplasmic reticulum, Golgi bodies, or lysosomes. The trophozoites inhabit the duodenum and jejunum. They are rarely passed in stools except in cases of florid diarrhea associated with giardiasis.

The cyst of *G. lamblia* measures 8–12 by 7–10 μm . Encystation of trophozoites takes place as they pass down the intestine, and it is the cyst stage that is generally excreted in the stools. *Giardia lamblia* cysts are resistant to 0.5% chlorinated tap water and are capable of surviving in cold water for months [for 2 months at 8°C, for 1 month at 21°C, and for 4 days at 37°C (Meyer and Jarroll, 1980)]. Cysts are destroyed by boiling water and heat greater than 59°C (Amin, 1979). *Giardia* require an optimum pH of 6.38–7.02 which is supplied by the duodenum and upper jejunum (Haiba, 1954).

Two criteria have been used to identify *Giardia* species: host specificity and morphological characteristics. Hegner (1926) believed that *Giardia* were host specific; Filice (1952) did not think that there was enough experimental evidence to believe so. It is recognized that prior *Giardia* infection may result in rejection of an attempted reinfection (Roberts-Thomson *et al.*, 1976). Past negative cross-infection results in animal models could be explained by this phenomenon of acquired immunity or poor selection criteria rather than absolute host specificity. For the last 85 years, *Giardia* researchers have been trying cross-transmission experiments between different species. *Giardia duodenalis* has been successfully transmitted from humans to puppies (Kessel, 1929; Padchenko and Stolyarchuk, 1970), kittens (Fantham and Porter, 1916; Kessel, 1929), and rodents (Armaghan, 1937; Goritskaya and Vrublerskaya, 1966; Haiba, 1956; Sharapov and Solov'er, 1977). Nauruzbaeva (1969) has shown that *G. duodenalis* can be transmitted from dog to mice, and Saxe (1950) in his study brought about the successful transmission of *G. duodenalis* from rat to hamster. Similarly, *G. muris* has been successfully transmitted from mouse to rat (Armaghan, 1937; Sebesteny, 1974; Simon, 1922) and puppies (Nauruzbaeva, 1969). Sebesteny (1974) showed that *G. muris* can be transmitted from rats to mice. Thus, it is clear that rigid host specificity does not exist. Animals can be infected with *G. lamblia* from man, and the reverse may be suggested to be true, as is evident from epidemiologic data of Barbour *et al.* (1976) that suggest that man may acquire *Giardia* infection from lower animals.

However, morphologic criteria may be employed for identifying various species of *Giardia*. Body measurements have not been found to be ideal for this purpose, since body dimensions of the parasite can change with the host's diet and nutrition, as observed by Tsuchiya (1931). All *Giardia* species have a median body, and variations in the size and shape of this structure have been used to divide this genus into groups. Median bodies are tubular structures that have some similarities to the ventral disk of the trophozoite and may bear some relation to it.

Filice (1952), having refuted host specificity of *Giardia* and use of body dimensions as means of classification, proposed the following species of *Giardia* based on morphology of the median body: *Giardia agilis* which has a long teardrop-shaped median body parallel to the long axis of the body, is long and narrow, and infects frogs and tadpoles; *Giardia muris* which has two small round median bodies situated in the center of the body and which infects mice, rats, and birds; and *Giardia duodenalis* which usually has a double, but occasionally single, median body resembling the claw of a claw hammer lying transversely across the organism and which infects humans, cats, dogs, and beavers.

In ultrastructural observations of *Giardia muris* in a mouse model (Nemanic *et al.*, 1979), endosymbiotic microbes 240–360 nm wide and 600–1400 nm long were seen. The endosymbionts were concentrated centrally. Seven humans having giardiasis underwent jejunal biopsy. The *G. lamblia* observed had all the cellular organelles seen in *G. muris* but no endosymbionts.

4. EPIDEMIOLOGY

Man is the host as well as a reservoir of *G. lamblia*. The infection is acquired from other humans or animal reservoirs including beavers, cats, dogs, etc. The mode of transmission is by the oral–fecal route through water, ice, beverages, food, and sexual activity.

Giardiasis is recognized all over the world, and Wolfe (1979) in his review of 670 cases noted that giardiasis can be contracted in at least 97 different countries. The disease is highly prevalent in Asia, Australia, Africa, western South America, Mexico, and the Soviet Union. Travel to these endemic areas carries the risk of acquiring *Giardia* infection.

Stated incidences of infestation vary below 1 and 30%. Infection is more frequent in children and is related to poor personal hygiene, poverty, and poor sanitation. Furthermore, estimates of prevalence vary according to the type of population group sample, e.g., field studies, hospital patients, speciality clinics, etc. A prevalence rate of 1% was found in retrospective analysis of approximately 4900 stool specimens (Uthman and Norton, 1969). A prevalence rate of 2% was found by Babb *et al.* (1971) in their study of 4400 stool specimens. The

prevalence of infection in the United States in 24 surveys of nearly 36,000 persons was 7.4% (Quinn, 1971). Petersen (1972) summarized the result of surveys on giardiasis in 21 countries and found that prevalence rates of 2–6% were common. In our hospital, the yield rate for *G. lamblia* on examination of stool samples is approximately 1%, being greater in select groups, for example, immunodeficiency patients (R. K. Chandra, unpublished data).

Giardiasis is seen in epidemic and endemic forms (Veazie, 1969). Twenty-three water-borne outbreaks have been reported in the United States since 1965 (Jacobowski and Hoff, 1979). In Aspen, Colorado, between 1965 and 1966, there was an outbreak of giardiasis in many ski resorts, and 11.3% of skiers were affected. In this case, it was found that the well water was contaminated by a leaking sewage pipe (Moore *et al.*, 1969). In 1972, there was an epidemic of giardiasis among 300 residents of Boulder, Colorado. The source of the epidemic was never found (Schultz, 1975).

Giardia is frequently acquired during travel to those areas of the world where the parasite is endemic. The Centers for Disease Control, Atlanta, examined questionnaires from 1419 tourists who had returned from the Soviet Union between 1969 and 1973. Of 1419 tourists, 502 were ill during the trip or shortly thereafter. Stool examinations were done in 306 of these tourists and showed *G. lamblia* in 91 cases, an incidence of 23% (Brodsky *et al.*, 1974).

In 1974, 34 out of 52 members of a sociology class who were on a camping trip came down with giardiasis after drinking water from a stream. Again in 1974, 69 out of 1100 residents were affected by this disease from the water supply (Schultz, 1975), and in the same year in Kentucky, five out of ten members of a farm family contracted giardiasis from water stored in an underground cistern (Brady and Wolfe, 1974). In 1977, there was another water-borne *Giardia* epidemic in Berlin, New Hampshire, where, in a 6-week period, there were 213 stool-positive cases of giardiasis. This was detected in the county hospital where no patient with *Giardia* in the stools had been seen in the 2 years prior to the epidemic (Lopez *et al.*, 1980).

Giardiasis can be a problem in day care centers and in institutions for the mentally retarded. Black *et al.* (1977) investigated an outbreak of giardiasis in three day care centers near Atlanta and found that 29–54% of children between 6 and 42 months had *G. lamblia* infection. These rates were significantly higher than 2% prevalence rate in age-matched neighborhood children who did not attend day care centers. Careful epidemiologic study of these cases indicated that the transmission of the parasite was directly from child to child.

In Canada, there have been two recent reports of *Giardia* epidemics: one involved two day care nurseries, and another epidemic was among inmates of an institution for the mentally retarded. Keystone *et al.* (1978) studied an epidemic of giardiasis in two day care nurseries in metropolitan Toronto and again established person-to-person spread of *Giardia* infection. In this study, the percentage

Table I
Frequency of *Giardia lamblia* Infection among Children in Two Day Care Nurseries and among Staff and Household Contacts of Infected Children, as Determined from Stool Testing^a

Group	Nursery A		Nursery B	
	No. tested	No. (and percent) infected	No. tested	No. (and percent) infected
Children	152	59 (39)	175	30 (17)
Staff	30	2 (7)	40	3 (8)
Household contacts of infected children	124/128	9 (7)	57/60	13 (23)

^aFrom Keystone *et al.* (1978).

of children infected in the two nurseries was 39% and 17%, respectively; infection spread to 7% and 23% of their household contacts (Table I). The authors noted that infection was more common between 1 and 3 years and that in nursery A 71% of children infected were of Canadian origin, although immigrants from India, Africa, southeast Asia, and the West Indies comprised 55% of the population of the nursery (Table II). These data point to relative differences in instances based on age and possible previous exposure.

This study confirms the observation of Roberts-Thomson *et al.* (1976) in animal models that prior infection with *G. lamblia* conferred partial immunity to rechallenge. It is possible that immigrant children might have been exposed to *G.*

Table II
Characteristics of Infected Individuals in Nursery A and Their Infected Household Contacts^a

Group	No. of individuals					
	Age (yr)		Sex ^b		Origin ^c	
	Mean	Range	Male	Female	Canadian	Immigrant
Children (<i>N</i> = 59)	2	0.75-4	31	28	42	17
Household contacts of infected children (<i>N</i> = 9)	30	27-43	4	5	8	1
Total (<i>N</i> = 68)	—	—	35	33	50	18

^aFrom Keystone *et al.* (1978).

^bNumbers in the two categories were not significantly different.

^cNumbers in the two categories were significantly different ($P < 0.01$).

lamblia previously, which might have provided a degree of protection not present in susceptible Canadian children. The age-specific attack rate of giardiasis in nursery A was 96% in children between 1 and 2 years, 26% in children between 2 and 3 years, and 80% in children 3 years old. The children in certain rooms had high rates of infection, whereas those in other rooms were unaffected. There were strong indications of person-to-person transmission of *G. lamblia*. In nursery A, low frequency of infection was noted in children less than 1 year which was explained by the fact that they were mostly in diapers and individual cribs and out of direct contact with other children. Children between 1 and 2 years were maximally infected as they were mobile and not toilet trained, and hence, person-to-person transmission was a possibility. The infection decreased with age as children were toilet trained and their personal hygiene improved. The virtual absence of infection in one of the rooms, amongst 2½- to 3-year-olds was explained on the basis of the teacher's desire to keep children's hands clean.

In a Manitoba residential school for the mentally retarded with a population of 250–275 students, there was an epidemic of giardiasis in 1978 (Naiman *et al.*, 1980). The parasitic infection rate in this institution was just under 50%, and *Giardia* comprised 24% of the infections. The mode of spread in this case was also from person to person.

5. RISK FACTORS

5.1. Age

Children are infected 3–4 times more often than adults. The clinical manifestation can be severe in children (Veghelyi, 1938; Cortner, 1959; Pugh and Newton, 1980). Meuwissen *et al.* (1977) have reported results of stool examinations done at the University Hospital, Nijmegen, on patients visiting outpatient departments between 1973 and 1976 with complaints of abdominal pain or diarrhea. In this study, giardiasis was found to be endemic, with a prevalence rate of 6%. Table III shows distribution by age of patients positive for *Giardia* on stool examinations carried out during the year 1976. From this table, it is evident that infection is 3–4 times higher in children between the ages 2 and 14 years than in adults.

5.2. Gastrectomy, Hypochlorhydria, and Achlorhydria

A breakdown in the gastric barrier increases the risk of infection with *Giardia*. Gastric resection or decrease in acidity makes the person more susceptible to giardiasis as the acid-bearing surface and its lethal contents are lost. Hass and Bucken (1967) studied 50 cases of giardiasis and found that 42% of them had

Table III
Age Distribution of *Giardia*-Positive Patients at University Hospital, Nijmegen^a

	Age (yr)										Total
	0	1	2-4	5-9	10-14	15-19	20-39	40-59	60+	Unknown	
No. of patients examined	18	45	138	165	83	47	505	394	132	152	1679
No. positive for <i>Giardia</i> cysts	0	4	23	34	12	2	25	15	1	4	120
Percent positive for <i>Giardia</i> cysts	0	9	17	21	14	4	5	4	1	3	7

^aFrom Meuwissen *et al.* (1977).

hypochlorhydria and 12% were achlorhydric. These authors suggested that disturbance of gastric acid production may facilitate *Giardia* infection. Yardly *et al.* (1964) studied six cases of *G. lamblia* infection and stated "perhaps gastrectomy renders a patient more prone to *Giardia* infestation, for it is conceivable that normal gastric function ordinarily provides a significant barrier to *G. lamblia* infestation."

5.3. Immunodeficiency

Patients with hypogammaglobulinemia and secretory IgA deficiency are more prone to infection with *Giardia* and particularly likely to have severe symptoms. Hermans *et al.* (1966) described seven cases of dysgammaglobulinemia with nodular lymphoid hyperplasia and found that six had *G. lamblia* in their stools. Hoskins *et al.* (1967) described six cases of giardiasis and malabsorption. In this study, the clinical syndrome was severe in two cases: one patient had hypogammaglobulinemia and another had IgA deficiency. Hughes *et al.* (1972) described six cases of primary hypogammaglobulinemia; of these, four had malabsorption and five had *G. lamblia* in their duodenal fluid. In a series reported by Ament and Rubin (1972), seven out of eight patients with hypogammaglobulinemia had gastrointestinal symptoms. *Giardia lamblia* was noted in stool specimens of all seven patients.

Zinneman and Kaplan (1972) examined ten cases of chronic giardiasis along with ten controls 3 to 9 months after the *G. lamblia* were eradicated with quinacrine. Serum immunoglobulin levels were normal in cases with giardiasis and controls. However, secretory IgA from duodenal fluid of the patients was significantly lower (mean 52.4 mg/gl) than in controls (mean 162.3 mg/dl). These results were questioned because of the methods used in intestinal fluid collection and immunoglobulin measurements (McClelland *et al.*, 1973.) Jones and Brown

(1974) measured immunoglobulins in the serum and intestinal fluid of nine giardiasis patients and 12 controls. The serum immunoglobulin concentrations and intestinal IgA and IgM levels were similar in the two groups; however, IgA levels were significantly higher in patients than in healthy individuals. This finding could not be explained except as a consequence of parasite infestation. The authors commented that "though giardiasis is very common in grossly immunodeficient patients, in otherwise healthy individuals, it is not related to unsuspected immunoglobulin deficiency." Naik *et al.* (1979) studied cell-mediated and humoral immune responses in ten cases of giardiasis and ten healthy individuals. There was no difference in immunologic functions in the two groups. In a recent study of 71 patients with selective IgA deficiency, stool examination was done in 31 cases, and four specimens were positive for *G. lamblia*, an incidence of 12.8% (Chandra *et al.*, 1982) contrasted with the presence of *Giardia* in about 1% of all stool samples examined.

Specific humoral response does occur in giardiasis, although its role in protective immunity is not clear. The subject of that resistance to giardiasis is beyond the scope of this review and has been discussed elsewhere (Stevens, 1981). Serum antibodies in giardiasis have been demonstrated by indirect fluorescent antibody test and by precipitin test. Ridley and Ridley (1976) demonstrated circulating antibodies against *G. lamblia* in 32 out of 36 cases (89%) of giardiasis associated with malabsorption but not in two cases without malabsorption. The test was negative in 17 controls but positive in 10 out of 34 cases (30%) of cryptic giardiasis. Vinayak *et al.* (1978) demonstrated a positive precipitin test in 90.9% of cases of giardiasis using an agar gel technique. In this study, there were 11 patients with giardiasis, 16 controls, and 15 patients with other parasitic infections. In both controls and patients with other parasitic infestation, the precipitin test was negative. Cell-mediated immunity also plays an important role in *Giardia* infestation. Athymic mice have a very severe infection which persists longer than that in mice with normal thymuses. Also, following giardiasis, the patients' resistance to reinfection has been found to increase (Smith and Wolfe, 1980). Some investigators have shown that after a few weeks of *Giardia* infection, the plasma cells in the gastrointestinal tract produce IgM to be followed later by production of IgA and IgG (Ridley and Ridley, 1976; Thompson *et al.*, 1977). Thompson *et al.* (1977) studied 17 cases of giardiasis diagnosed by jejunal biopsy; 13 biopsy sections were examined under direct immunofluorescence. Three cases had a significant increase in IgM, seven had increases in IgM and IgA, one had increases in IgA and IgG, one had increased IgM, IgA, and IgG, and one had increased IgA. In this report, both patients in whom IgG was elevated had severe mucosal lesions. The nine patients studied by Jones and Brown (1974) had increased intestinal IgG, but none had jejunal biopsy. Thus, one remains unaware of the severity of lesion in these patients and cannot generalize that immunoglobulin-bearing cells in the intestine of patients with

giardiasis in mild to moderate cases are predominantly IgM type and, in severe cases, are of IgG type.

5.4. Homosexuality

There is a higher incidence of giardiasis in homosexual men, and it has been suggested that *Giardia* can be transmitted sexually. Meyers *et al.* (1977) described six male homosexuals with symptoms of giardiasis, four of whom were members of the same household. At least four out of six had had oral–anal contact with other infected members of the group, and direct feco–oral transmission was proposed as the mode of transmission.

Schmerin *et al.* (1978) reviewed 133 cases of giardiasis seen over a 5-year period in a New York hospital. The study included all patients over the age of 15 years at the time of infection. Medical records of all these patients were evaluated for sex, travel history, sexual preferences, associated diseases, and history of venereal diseases. In the study there were 89 males and 44 females. The results are shown in Table IV: 75% of men and 94% of women had recently traveled to an endemic area; two male travelers were homosexuals; four patients (three males and one female) had associated immunodeficiency; 19 males were nontravelers, and all were homosexuals. The authors conclude with the note that giardiasis should be added to the list of venereally transmitted diseases.

5.5. Enteral Bacterial Infection

Bacterial infection of the small intestine may enhance susceptibility to giardiasis. Ingram *et al.* (1966) made a controlled study in a large population of children with diarrhea for the presence of enteropathogenic bacteria and parasites. *Giardia lamblia* was found in 31.9% of diarrheal cases and in 19.1% controls. Enteropathogenic bacteria were associated with *G. lamblia* in 9% of cases of diarrheal diseases but in none of the control group. Tomkins *et al.* (1978) noted that concomitant enterobacterial colonization of the small intestine was associated with increased severity of mucosal lesion and malabsorption in

Table IV
Contributing Factors in *Giardia* Infection

Patient groups	Men (N = 89)		Women (N = 44)	
	Cases	Percent	Cases	Percent
Travelers	67	75.3	41	93
Nontravelers	19	21.3	2	5
Immunodeficiency	3	3.3	1	2

their 9 of 14 cases of giardiasis. The presence together of bacteria and *Giardia* may result in a more symptomatic syndrome.

5.6. Malnutrition

The interaction between intestinal parasites and nutritional status complex, one aggravating the other. Malnutrition decreases host resistance (Chandra and Newberne, 1977; Chandra, 1980), and gut infestations have significant nutritional implications (Solomons and Keusch, 1981). Besides immunodeficiency, hypochlorhydria and bacterial contamination of the upper gut may promote infection with *G. lamblia*. *Giardia* is a common parasite in children with protein–energy malnutrition. The role of selective nutrient deficiencies as synergistic or antagonistic factors in giardiasis has not been studied. Response to antiparasitic therapy also may be conditioned by nutritional status of the host. For example, in his study of 11 patients with parasitic infections, two of whom had giardiasis, Mayoral *et al.* (1966) reported improvement and correction of malabsorption following treatment of associated protein malnutrition. It is suggested that *Giardia lamblia*, bacteria, and protein–calorie malnutrition operate synergistically to produce a severe clinical picture, although the exact nature of the association and the pathogenic factors are complex and poorly understood.

5.7. ABO Blood Groups

Giardiasis has been noted to be associated with increased frequency of blood group A and decreased frequency of blood group O. Barnes and Kay (1977) studied 62 children diagnosed as having giardiasis by either examination of stool or duodenal fluid; 61% of these children had blood group A as compared to 39% in the control population. Zisman (1977) examined 201 children between the ages of 4 months and 14 years with varied gastrointestinal complaints; 55 children (27%) were diagnosed to have giardiasis by microscopic examination of the stools. In the *Giardia* group, 44.93% had blood group A, and 17.15% had

Table V
Blood Group Distribution among Children with Gastrointestinal Complaints with and without *Giardia*^a

Blood group	Controls	Giardiasis
O	3804 (47.94%)	12 (17.15%)
A	2767 (34.86%)	31 (44.93%)
B	1112 (14.01%)	9 (21.43%)
AB	254 (3.19%)	3 (15.00%)

^aFrom Zisman (1977). The groups differ significantly ($z^2 = 11.0$; $P < 0.025$; $df = 3$).

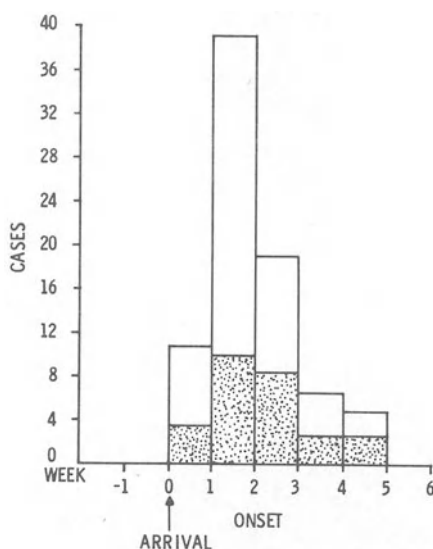


Figure 2. Onset of symptoms related to arrival in endemic area. The symptomatic patients are shown as stippled columns, and asymptomatic carriers of *Giardia* as open columns. From Walzer *et al.* (1971).

blood group O. In controls, the figures were 34.86% and 47.94%, respectively, for groups A and O (Table V).

5.8. Travel to Endemic Areas

Last, but not least, is the risk associated with travel to those parts of the world where *Giardia* is endemic. In 1970, two groups of American tourists suffered from giardiasis on a visit to Leningrad, Soviet Union. In the first group, 23 out of 80 members (28.8%) were ill; *G. lamblia* was recovered from stool specimens of nine patients and two asymptomatic persons. In the second group, 84 out of 164 members (51.2%) were ill, and *G. lamblia* was found in stool specimens of 23 patients and three asymptomatic persons. Water was implicated as the source of infection in this outbreak, the epidemic curve of which is shown in Figure 2 (Walzer *et al.*, 1971).

6. PATHOGENESIS

The infection is contracted by drinking contaminated water and beverages or eating contaminated food. In the upper bowel, excystation takes place, and trophozoites are released. The trophozoites attach themselves to the bases of

microvilli in the duodenum and upper jejunum by means of suction hooks present on their ventral surface.

In the classic ultrastructural study by Owen *et al.* (1979), *G. muris* trophozoites were found to colonize the proximal 25% of the intestine of infected immunocompetent mice, to microvilli of columnar cells near the base of villi, and over Peyer's patches wedged into furrows in the epithelial surface or lodged in mucus within the unstirred layer (Figures 3–7). The parasite did not attach itself to M-cells which are involved in uptake of both particulate and soluble antigens from the lumen into the subepithelial lymphoid tissue. The density of trophozoite colonization correlated with cyst excretion in the stools.

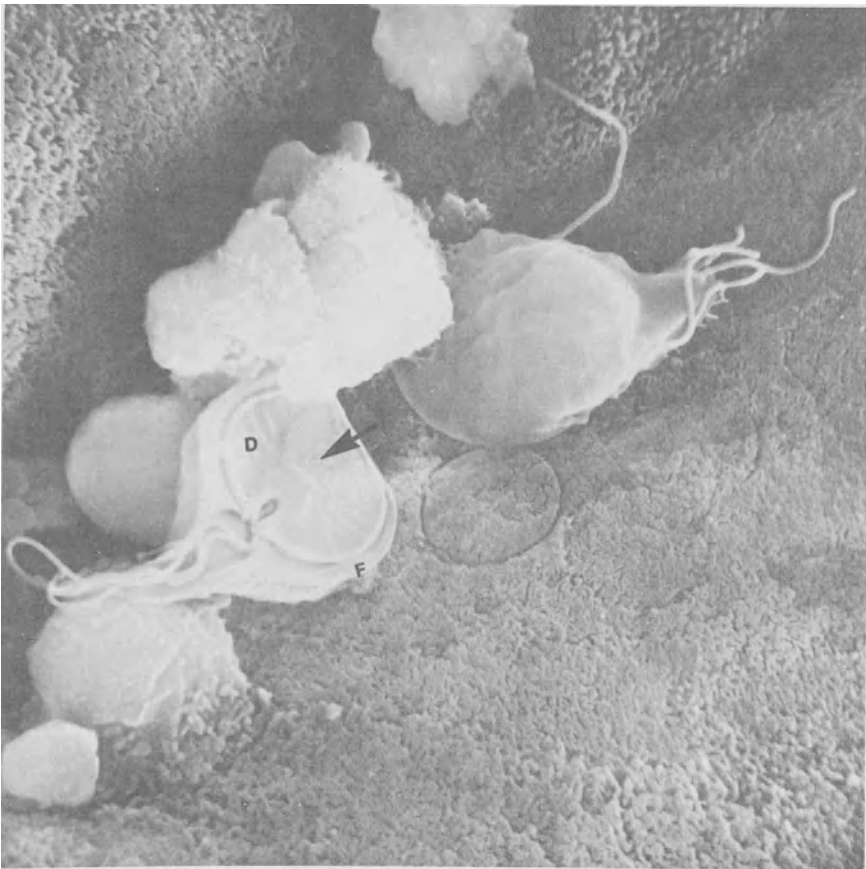


Figure 3. Dorsal and ventral aspects of *Giardia* trophozoites. Ventral surface exposes adhesive disk (D), cytoplasmic flange (F), naked area (arrow), and flagella. A circular adhesive disk mask is adjacent to the adherent trophozoite. Reduced to 40% from original magnification of $\times 8026$. From Owen *et al.* (1979).



Figure 4. Circular deformation in columnar cell microvilli. Overlapping disk marks indicate detachment and reattachment rather than grazing by trophozoites ($\times 1008$). From Owen *et al.* (1979).

Clinical symptoms do not correlate with worm load. A small number, as few as ten, can produce symptoms, and at least 100 cysts are required to guarantee infection. In an experiment conducted by Rendtorff (1954), 21 out of 40 (52.5%) men exposed to *G. lamblia* became infected. None exposed to one cyst became infected; 22 men received 10–25 cysts, and eight (36.4%) became infected; all who received 100 cysts or more became infected.

Early in the infection, patients may have numerous trophozoites in the upper



Figure 5. Villi in the mouse jejunum. *Giardia* trophozoites adhere to microvillus surfaces near bases of villi, wedge into furrows, or lie in mucus. No adhesive marks are seen on the villus apices. Reduced to 80% from original magnification of $\times 708$. From Owen *et al.* (1979).

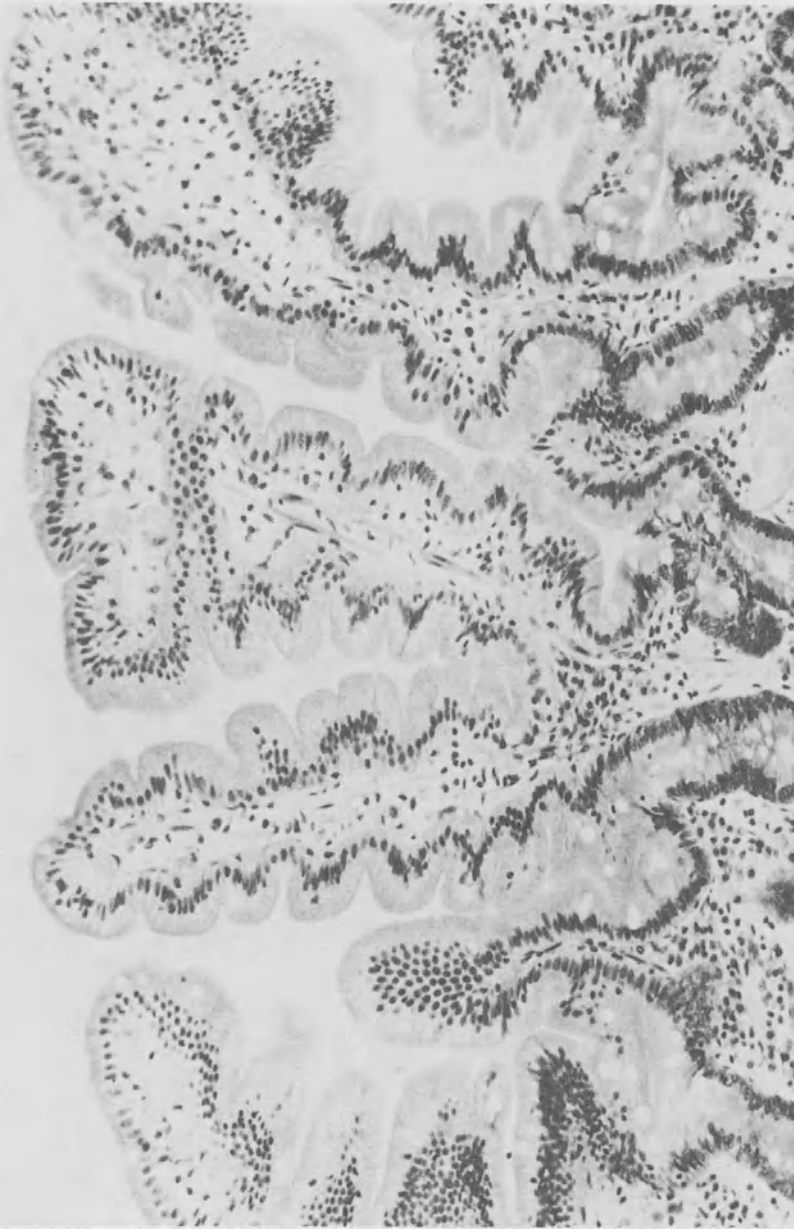


Figure 6. Jejunal histology in giardiasis. An almost normal jejunum with early grade 1 changes signified by edema in the epithelial cells in some areas causing upward displacement of the nuclei. There is also edema of the lamina propria in one villus. Courtesy of Dr. D. S. Ridley.

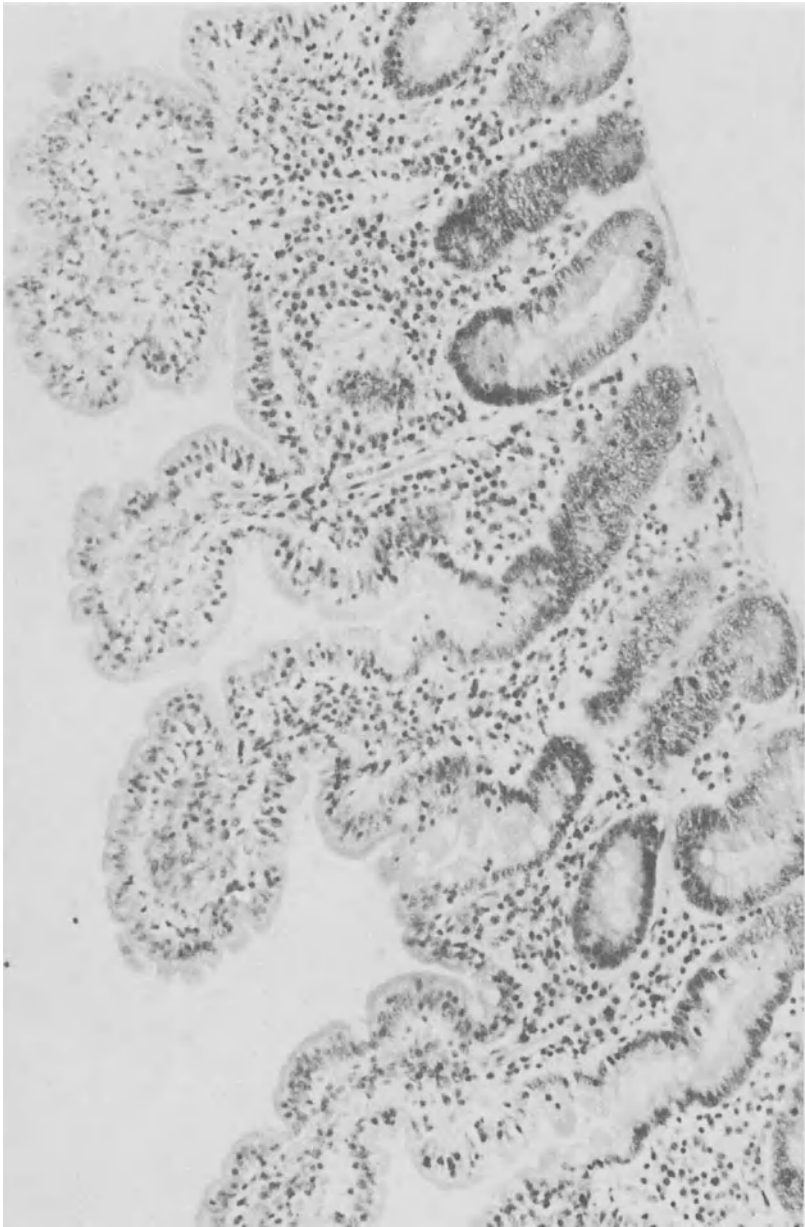


Figure 7. Grade II changes with a heavy infiltrate of chronic inflammatory cells in the lamina propria and some lymphocytes in the epithelium. Courtesy of Dr. D. S. Ridley.

intestine but no cysts in the feces (Jokipii and Jokipii, 1977). Some patients pass large numbers of cysts in stools regularly, some pass low numbers, and others pass variable numbers of cysts in a cyclic fashion (Danciger and Lopez, 1975).

The trophozoites prefer the upper small intestine for unknown reasons. It has been suggested that alkaline pH (Haiba, 1954), nutritional factors, or brush border receptors might have some role to play (Owen *et al.*, 1979).

The exact mechanism by which *G. lamblia* exert their pathogenicity is not known, but several theories have been put forward. In 1939, DeMuro postulated that *G. lamblia* invades the pancreatic duct and causes pancreatic insufficiency. Later, Veghelyi (1939) suggested that *G. lamblia* by their sheer multitude caused mechanical blockage of the brush border and hence impeded the absorption of nutrients, resulting in diarrhea. This theory, however, cannot explain the cause of symptoms in some patients with small number of *G. lamblia*. Also, it is known that organisms do not cover the brush border but remain unattached in the mucus layer at bases of microvilli (Yardly *et al.*, 1964). Another theory put forward is mechanical irritation of the microvilli. The organisms attach to the mucosa of the duodenum and jejunum by means of suction hooks. This causes irritation of the microvilli and direct injury to their fuzzy coat in the acute stage of the disease when there are many organisms. Although microvillus changes are reversible in most of the patients following treatment, in some cases there is evidence of malabsorption of disaccharidases, peptide hydrolysate, and vitamin B₁₂, the absorption and digestion of which are dependent on the integrity of microvilli (Erlandsen and Chase, 1974; Wolfe, 1978).

Production of toxic substances by organisms has been suggested as one of the pathogenetic mechanisms of *G. lamblia*; however, culture filtrates do not cause intestinal damage in the rabbit (Duncombe *et al.*, 1978).

Malabsorption in giardiasis may be caused by caterial overgrowth and bile salt deconjugation (Yardly *et al.*, 1964; Leon-Barua and Lumbreas-Cruz, 1968). In the study by Tandon *et al.* (1977), bile salt deconjugation was recorded in 14 of the 17 patients, and significant bacterial growth was observed in 48% of the patients. None of the control group cases had either bile salt deconjugation or significant bacterial overgrowth in their jejunal aspirates.

Zamcheck *et al.* (1963) proposed the theory of competition for nutrients as the cause of malabsorption in giardiasis. Gupta and Mehta (1973) noted reduced pancreatic lipase and trypsin in patients with *Giardia* infection. There was a significant correlation between fecal fat and pancreatic lipase which could explain steatorrhea. Chawla *et al.* (1975) noted decreased duodenal trypsin activity in their patients with giardiasis which returned to normal following treatment.

6.1. Mucosal Invasion and Injury

Electron microscopic studies by Brandborg *et al.* (1967) in ten patients with documented giardiasis revealed mucosal invasion in six patients with diarrhea

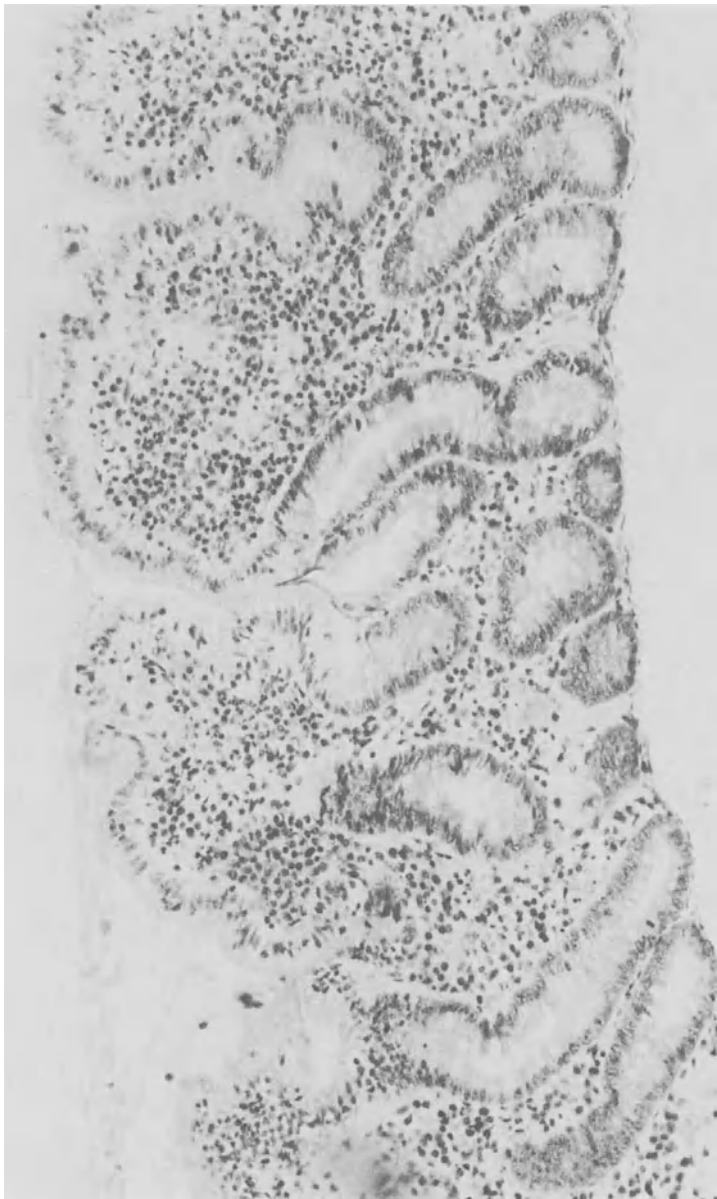


Figure 8. Grade III jejunal mucosa of a patient with giardiasis is a more advanced stage of grade II with greatly swollen, bulbous villi. Courtesy of Dr. D. S. Ridley.

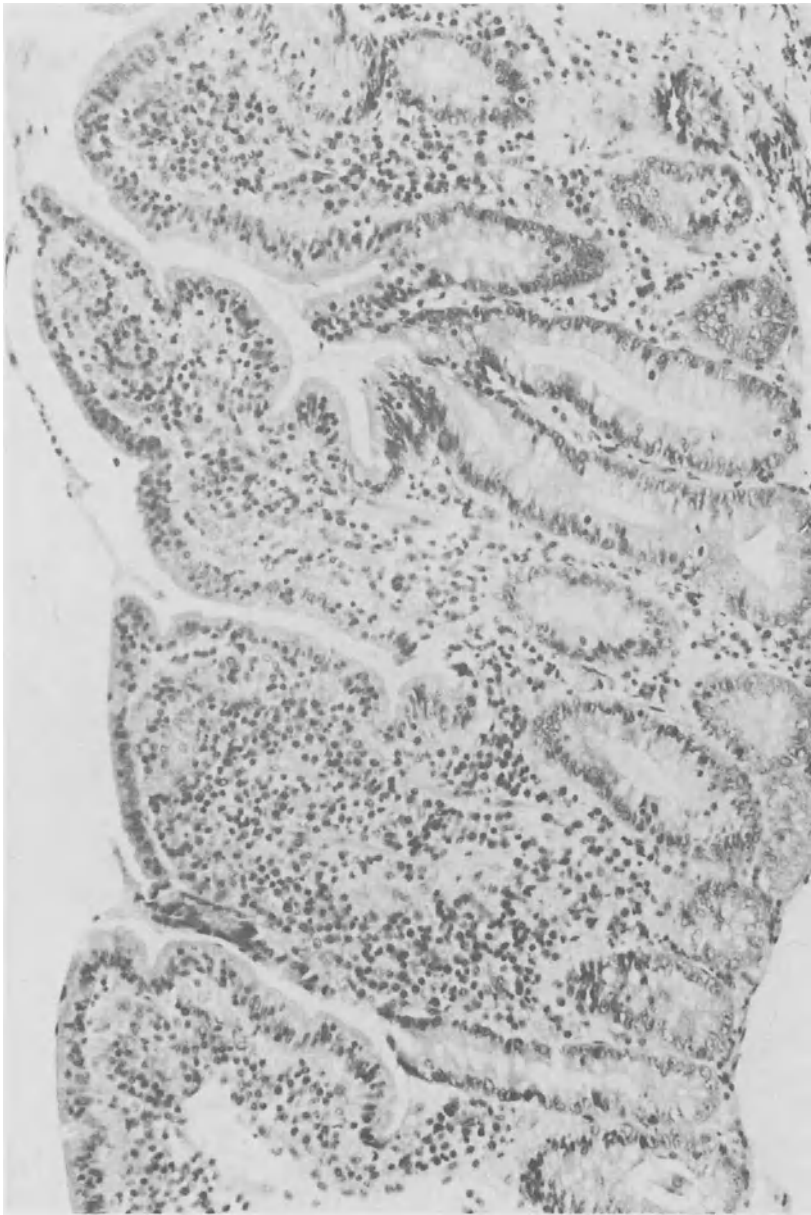


Figure 9. An early grade IV mucosa indicated by flattening of the tips of the villi. In more advanced lesions, there is almost complete fusion of the villi. Courtesy of Dr. D. S. Ridley.

and trophozoites in stools. Morecki and Parker (1967) also reported a case of giardiasis with mucosal invasion which improved after treatment. Mucosal lesions in patchy distribution were seen in serial sections of 384 jejunal biopsies taken from eight patients by Ament and Rubin (1972). Mucosal injury is perhaps the most important cause of *Giardia*-induced malabsorption, with mechanical interference and enzyme alterations contributing significantly (Sheehy and Holley, 1975); however, invasion of mucosa and submucosa is infrequently documented, and neither this finding nor changes in microvilli correlate with severity of symptoms (Wolfe, 1979).

A possible interrelation between *G. lamblia* and jejunal colonization with *Candida albicans* has been suggested (Naik *et al.*, 1978).

Strains of this protozoa may vary in their pathogenicity, and it is possible that *Giardia* acts synergistically with concomitant viral, bacterial, or fungal infections to increase the severity of infection, mucosal damage, and clinical manifestations (Wolfe, 1979).

Pathological findings in giardiasis include variable changes in intestine ranging from normal morphology to spruelike changes (Figures 6–9) (Yardly *et al.*, 1964; Hoskins *et al.*, 1967; Duncombe *et al.*, 1978). The abnormalities seen include decreased villus–crypt ratio, increased epithelial cell turnover, local acute and chronic inflammation, round cell infiltration, and hyperplasia of lymphoid nodules. The severity of morphological changes correlates with the clinical picture in some patients (Wright *et al.*, 1977; Duncombe *et al.*, 1978), but this is not invariable. Fluorescent antibody titer shows a rough correlation with severity of intestinal lesions (Ridley and Ridley, 1976). Mayer and Radulescu (1979) have suggested a hypersensitivity reaction as the cause of increased intra-epithelial cell turnover as had previously been observed and postulated by Ferguson *et al.* (1976), Wright and Tomkins (1977), and MacDonald and Ferguson (1978).

7. CLINICAL MANIFESTATIONS

The incubation period of the disease is usually 1–3 weeks (Amin, 1979). In another study (Walzer *et al.*, 1971), the average incubation period was 15 days. Rendtorff and Holt (1954) carried out experiments on human volunteers and found the mean incubation period to be 13.1 days.

The infection may be acute, subacute, or chronic. The acute disease lasts 1–4 weeks and is characterized by explosive, foul-smelling, frothy, watery stools without blood or pus which float in toilet water. There is also weakness, abdominal cramps and distension, foul-smelling flatulence and/or belching, anorexia, nausea, vomiting, and low-grade fever. Diarrhea, however, is the most common symptom.

The acute infection commonly resolves spontaneously, and organisms disappear from the feces. Some patients become carriers for a period without symptoms, and still others may continue to have subclinical malabsorption. In the acute stage, giardiasis has to be differentiated from bacillary and amoebic dysentery, acute gastroenteritis, salmonellosis, and bacterial food poisoning (Smith and Wolfe, 1980; Wolfe, 1979). In subacute infection, the symptoms are mild to moderate and may persist for months to years. The chronic infection is usually mild and may last for years. Children with chronic infection may present with failure to thrive and are stunted in growth (Kidney and Holland, 1967). The subacute and chronic infections have to be differentiated from malignancy, peptic ulcer, hiatal hernia, and gall bladder disease. Table VI shows the frequency of different symptoms as found by Walzer *et al.* (1971) in their study of 107 cases of giardiasis.

On being infected with *G. lamblia*, the patients may fall into any one of the following three groups:

1. They may be completely asymptomatic. Veazie (1969) in his study found 50% of the patients to be asymptomatic. This is the largest group.
2. Patients may have the typical disease with mild-moderate diarrhea and other symptomatology which persists for about 6 weeks followed by spontaneous recovery.
3. The third group of patients are those with severe disease, with profound diarrhea, nutrient loss, acute abdominal distension and cramps, and electrolyte disturbance.

On one end of the spectrum, there are patients who are asymptomatic, and on the other end, there are those who have severe malabsorption. Lactose intolerance, hypocarotenemia, reduced D-xylose absorption, steatorrhea, and abnormal vitamin A and B₁₂ absorption have been described in patients with giardiasis. Yardley *et al.* (1964) noted significant steatorrhea in five of the six patients with giardiasis. Hoskins *et al.* (1967), in their study of six patients, noted defects in carbohydrate absorption in five individuals, impaired mucosal disaccharidase activity in one, and mild steatorrhea (two patients). Wright *et al.* (1977) studied 40 symptomatic giardiasis patients; 29 had malabsorption, 23 impaired D-xylose absorption, 20 low vitamin B₁₂ absorption, and 15 steatorrhea. Similar findings were reported by Ament and Rubin (1972). Palumbo *et al.* (1962) and Kotcher *et al.* (1966) did not observe steatorrhea in their patients with giardiasis. Low vitamin B₁₂ absorption has been demonstrated by Conn *et al.* (1968) and Cowen and Campbell (1973). Mahalanabis *et al.* (1979) showed impaired vitamin A absorption in 12 patients with giardiasis which improved following treatment.

Rare clinical and laboratory findings include eosinophilia (Welch, 1943), urticaria (Harris and Mitchell, 1949), uveitis (Carroll *et al.*, 1961), bron-

Table VI
Clinical Features of Illness in Patients with Giardiasis^a

Symptoms	Percent of all cases (N = 107)	Percent of cases with <i>Giardia lamblia</i> in stool (N = 32)
Nausea	41.1	59.4
Vomiting	15.9	34.4
Cramps	49.5	59.4
Belching	23.8	30.4
Flatulence	56.0	56.5
Diarrhea	81.3	71.9
Blood in stool	—	—
Mucus in stool	8.3	4.4
Greasy, foul- smelling stool	42.9	52.2
Anorexia	41.1	56.2
Fatigue	74.8	87.5
Fever	11.9	17.4

^aFrom Walzer *et al.* (1971).

chopulmonary symptoms and signs (Fossati, 1971), and joint manifestations (Goobar, 1977). In these observations, once *G. lamblia* was eradicated, the symptoms disappeared.

8. DIAGNOSIS

Prompt diagnosis is important because symptoms are reversible once infection is eradicated by appropriate treatment (Babb *et al.*, 1971; Ament, 1972); however, sometimes giardiasis may be difficult to diagnose when symptoms are nonspecific and stool examination is negative (Hoskins *et al.*, 1967; Brandborg *et al.*, 1967).

8.1. History

As in any other aspect of clinical medicine, history is important. History of travel to endemic areas of the world might lead an agile physician to look for *Giardia* cysts in stool specimens. If necessary, one should not hesitate to go into sexual attitudes and preferences to find out whether a person is homosexual or not (Schmerin *et al.*, 1978).

Giardiasis cannot be diagnosed on the basis of symptoms which are varied,

and none of which are pathognomonic of the disease. For definitive diagnosis, one has to demonstrate trophozoites or cysts in the stool or duodenal specimens.

8.2. Stool Examination

Wet preparations of stools are made. One may see trophozoites if the patient has acute diarrhea and the stool specimen is examined without delay. The slide has to be stained with iodine to observe cysts. It is advisable to use one of the fecal concentration methods—zinc floatation (Faust *et al.*, 1939), formal–ether concentration technique (Allen and Ridley, 1970)—for achieving higher yield in stool examinations. At least three specimens of stools should be examined at intervals of 36–48 hr, as stools may be negative for *G. lamblia* in early stages of infection (Jokipii and Jokipii, 1977) and some patients pass the parasite in a cyclic fashion (Danciger and Lopez, 1975). Wolfe (1979) reported a 76% yield with the first stool specimen, 90% with the second, and 97% with the third. However, others report that the yield of the stool examination is not more than 50% (Carswell *et al.*, 1973; Burke, 1975).

Barium, antibiotics, antidiarrheals, certain enema preparations, and oil laxatives can temporarily mask the presence of the parasites, and their use should be deferred until stool examinations are completed (Wolfe, 1978). If the stool examination is repeatedly negative, and one has strong suspicion about giardiasis, then any of the following tests may be done.

8.3. Duodenal Aspiration

A double-lumen tube is passed into the duodenum under fluoroscopic control. Fluid is aspirated from the intestine and examined under a microscope. By this method the yield is higher than repeated stool microscopic examination.

8.4. Enterotest

Developed in 1970, this test consists of swallowing a weighted gelatin capsule lined in its interior with silicone rubber. A 1-m nylon thread is packed in the capsule with one end protruding through the hole. The free end of the yarn is held, and the patient swallows the capsule. In the stomach, the gelatin capsule dissolves leaving the weighted rubber bag which is carried by peristalsis to the duodenum. The free end of the string is taped to the face for 3–4 hr. The patient is allowed to take only water during the procedure. When the thread is removed, the distal 30 cm or more is bilestained. Mucus from this portion is scraped out, and slides are made to observe *G. lamblia*. This is a simple, accurate, and inexpensive test.

Duodenal aspiration and enterotest techniques were evaluated by Bezjak

(1972) in 51 patients with giardiasis. Both methods demonstrated *Giardia* with equal frequency, 82.2% by intubation and 81.6% by the capsule method. Rosenthal and Liebman (1980) studied 28 children with chronic diarrhea. Stool for *G. lamblia* was negative in all children; however, in five patients duodenal juice and enterotest revealed *G. lamblia*.

8.5. Small Intestine Biopsy

This is the most reliable method of finding *G. lamblia*. In children, the pediatric Crosby capsule is preferred. The procedure is invasive, time consuming, and inconvenient for the patient, but the yield is very high. Evaluation of biopsy sections is cumbersome, as one has to look carefully through many sections, and sometimes it becomes difficult to interpret, particularly if there is much cellular debris present. Brandborg *et al.* (1967) studied ten patients with giardiasis. The biopsy of the jejunum revealed *G. lamblia* in six of the ten cases examined. In four jejunal biopsies, no *Giardia* could be detected, although cysts were detected in stools. Barbieri *et al.* (1970) were able to identify parasites in biopsies of all patients whose duodenal aspirates were positive for *G. lamblia*. Ament (1972) compared the three months, stool examination, mucus smear, and biopsy, in his five patients with giardiasis. Mucus smear and biopsy both were positive in three cases in which stool examination was negative. Kamath and Murugaso (1974) compared all four methods, stool examination, duodenal aspirate, mucus smear, and biopsy, in their 21 patients who either had diarrhea for more than 2 weeks, malabsorption, or gastroenteritis. Out of 21 children, 13 had chronic diarrhea, two had malabsorption, and six had acute gastroenteritis. Giardiasis was confirmed by one or more of the diagnostic methods in 12 out of 21 patients. Stool examination was positive in six patients with acute gastroenteritis. In all these patients, trophozoites were seen. Duodenal aspirate was positive for trophozoites in ten patients, four with positive stool and six with negative stool examination. Biopsy revealed trophozoites in all 12 patients. Mucosal impression smears were positive for trophozoites in all 12 cases. When doing a biopsy, the mucus on surface of the section should be separately smeared and examined for *G. lamblia*. This is quicker and simpler in diagnosing giardiasis than looking at the biopsy, because the smear takes less time to prepare, and trophozoites are seen more clearly (Ament, 1972).

8.6. Serologic Tests

These methods have been used recently. Ridley and Ridley (1976) reported a positive indirect fluorescent antibody test using *Giardia* cysts as antigen, and Vinayak *et al.* (1978) demonstrated positive precipitin test by an agar gel method.

8.7. Therapeutic Trial

Occasionally, one may be faced with a situation in which all tests done to demonstrate *G. lamblia* have been negative but there is a strong suspicion of infection on clinical and epidemiologic grounds. In such cases, it is best to give a trial of an effective anti-*Giardia* drug, and clinical recovery in such instances will confirm the existing suspicion regarding infection and at the same time cure the patient (Wolfe, 1975).

9. TREATMENT

There are three drugs available in North America for treatment of giardiasis. Tinidazole and nimrazole have been used in other countries with success (Anderson *et al.*, 1972; Levi *et al.*, 1977). However, these drugs are not licensed for use in the United States. The treatment results are very fruitful provided the physician keeps a high index of suspicion and institutes appropriate therapy without delay. The three drugs currently in use are quinacrine hydrochloride (Atabrine®), metronidazole (Flagyl®), and furazolidone (Furoxone®).

9.1. Quinacrine Hydrochloride

It is the treatment of choice for adults and older children (Smith and Wolfe, 1980). Most workers report a cure rate of 90–95% (Wolfe, 1975; Kavousi, 1979). However, Wright *et al.* (1977) were able to cure only five out of eight patients (63%) using this drug. Results are good in children. Powell (1956) and Cortner (1961) found this drug to be highly effective in children, with minimal side effects. However, quinacrine is a bitter drug, and young children do not tolerate it well. In five out of six patients, this medication had to be discontinued because of headache, vomiting, and intestinal upset (Wolfe, 1979). Dose for those <8 years of age is 2 mg/kg three times daily, and for individuals >8 years it is 100 mg three times daily. The treatment should be continued for a week.

9.1.1. Side Effects

Side effects include anorexia, nausea, vomiting, headache, dizziness, and yellow discoloration of urine (Wolfe, 1975; Kavousi, 1979). Other side effects include exfoliative dermatitis, discoloration of tears, sclera, and semen, skin rash, and toxic psychosis (Amin, 1979; Smith and Wolfe, 1980).

9.1.2. Contraindications

This medicine should not be used in patients with psoriasis. The drug should be avoided with alcohol and promaguin (Smith and Wolfe, 1980).

9.2. Metronidazole

This drug is frequently used but is less effective than quinacrine. Ament (1972) cured four out of five children using this drug for 10 days. Anderson *et al.* (1972) were able to cure only 71% of patients with giardiasis. Wolfe (1975) has reported a cure rate of 86%. Wright *et al.* (1977) used metronidazole in a single 2.0-g dose for 3 successive days and achieved a parasitological cure rate of 91%. In Finland, Jokipii and Jokipii (1978) reported a cure rate of 93% using metronidazole, 200 mg three times daily for 1 week and repeating the same regimen after 7 days. This drug has not been approved by the U.S. Food and Drug Administration for treatment of giardiasis (Wolfe, 1979).

9.2.1. Dose

The dose for those <8 years old is 5 mg/kg three times daily for 10 days; that for patients >8 years is 250 mg three times a day for 10 days.

9.2.2. Side Effects

Nausea, vomiting, metallic taste in the mouth, headache, adverse disulfiram-like reaction when used with alcohol, dark coloration of urine, and monilial infection of the vagina or gastrointestinal tract have been noted (Wolfe, 1975; Kavousi, 1979). Metronidazole has been found to be carcinogenic and teratogenic in rodents (Rustia and Shubik, 1972) and mutagenic in bacteria (Voogd *et al.*, 1974) and hence is contraindicated in pregnancy.

9.3. Furazolidone

It is the drug of choice in infants and young children. This is the only anti-*Giardia* drug available in suspension form. Wolfe (1979) reported parasitological cure in 24 out of 31 (77%) children treated with furazolidone.

9.3.1. Dose

The use dose is 6 mg/kg per day for 1 week.

9.3.2. Side Effects

Side effects of furazolidone are not uncommon. Nausea, vomiting, diarrhea, and fever may occur. Rarely, hypersensitivity reaction (urticaria), hypotension, and serum sickness can be seen. In glucose-6-phosphate dehydrogenase-deficient patients, this drug can cause mild reversible hemolysis. Furozone is a monoamine oxidase inhibitor and hence can precipitate a disulfuramlike reaction with alcohol.

9.4. Comparative Evaluation

Comparative usefulness of various anti-*Giardia* drugs has been studied by a number of workers. Levi *et al.* (1977) compared the relative efficacy of furazolidone, nimorazole, metronidazole, and tinidazole. In this study, the lowest cure rate was for furozolidone (71.7%) and the highest for tinidazole (96.7%). Gazder and Banerjee (1977) also preferred tinidazole to metronidazole in their study.

In another study, metronidazole was found to be superior to furazolidone (Singh *et al.*, 1977). There are other drugs that have been noted to have anti-*Giardia* effect. Hutchinson *et al.* (1975) in their clinical trial of mebendazole noted that out of 85 children, 25 had *G. lamblia* cysts in their stools, and 10 were cured (40%). Anti-*Giardia* activity of erythromycin has also been reported in the literature (Matthews and Chaudry, 1972; Nash, 1976).

Occasionally, the drugs may fail to clear *G. lamblia*. Weiss *et al.* (1977), in their study of giardiasis in Minnesota, noted that *G. lamblia* cysts persisted in many patients who had repeated courses of quincrine and metronidazole. The reason for this is not known, but the possibility of resistant strains of *G. lamblia* has to be entertained.

10. PREVENTION

There is no chemoprophylactic anti-giardial drug in the market. The best way to avoid this disease is to boil drinking water for at least 10 min. Uncooked or peeled vegetables and fruits should be properly treated prior to ingestion. Food-borne transmission is known, so cold plates should be avoided in endemic areas, and hot meals should be preferred. It has been noted that even after such elaborate precautions, people can contract *G. lamblia* when they visit an endemic area for a few days.

11. CONCLUDING REMARKS

Giardia has emerged as an intestinal parasite with significant health consequences in both under-developed and industrialized countries. In recent years, the organism has elicited considerable interest, and studies have been conducted to evaluate the prevalence, nutritional consequences, and diagnostic approach to *G. lamblia* infection. The host immune response to *Giardia* and means of boosting it are being investigated. It is evident that the last word on this interesting parasite of man and animals remains to be written.

REFERENCES

- Alexeieff, A., 1914, Notes protistologiques, *Zool. Anzeiger* 44:193–213.
Allen, A. V. H., and Ridley, D. S., 1970, Further observations on the formal–ether technique for faecal parasites, *J. Clin. Pathol.* 23:545–546.

- Ament, M. E., 1972, Diagnosis and treatment of giardiasis, *J. Pediatr.* 80:633–637.
- Ament, M. E., and Rubin, C. E., 1972, Relation of giardiasis to abnormal intestinal structure and function in gastrointestinal immunodeficiency syndromes, *Gastroenterology* 62:216–226.
- Amin, N., 1979, Giardiasis, a common cause of diarrheal disease, *Postgrad. Med.* 66:151–156.
- Anderson, T., Forssell, J., and Sterner, G., 1972, Outbreak of giardiasis: Effect of a new anti-flagellate drug, Tinidazole, *Br. Med. J.* 2:449–451.
- Armaghan, V., 1937, Biological studies on the *Giardia* of rats, *Am. J. Hyg.* 26:236–258.
- Babb, R. R., Peck, O. C., and Vescia, F. G., 1971, Giardiasis: A cause of traveller's diarrhea, *J.A.M.A.* 217:1359–1361.
- Barbieri, D., DeBrito, T., Hoshino, S., Nascimento, O. B., Martins Campos, J. V., Quarente, G., and Marcondes, E., 1970, Giardiasis in childhood: Absorption tests and biochemistry, histochemistry, light and electron microscopy of jejunal mucosa, *Arch. Dis. Child.* 45:466–472.
- Barbour, A. G., Nichols, C. R., and Fukushima, T., 1976, An outbreak of giardiasis in a group of campers, *Am. J. Trop. Med. Hyg.* 25:384–389.
- Barnes, G. L., and Kay, R., 1977, Blood groups in giardiasis, *Lancet* 1:808.
- Bezjak, B., 1972, Evaluation of a new technique for sampling duodenal contents in parasitologic diagnosis, *Am. J. Dig. Dis.* 17:848–850.
- Black, R. E., Dykes, A. C., Sinclair, S. P., and Wells, J. G., 1977, Giardiasis in day care centers: Evidence of person to person transmission, *Pediatrics* 60:486–491.
- Blanchard, R., 1888, Remarques sur le Mega storne intestinal, *Bull. Soc. Zool. Fr.* 30:18–19.
- Brady, P. G., and Wolfe, J. C., 1974, Waterborne giardiasis, *Ann. Intern. Med.* 81:498–499.
- Brandborg, L. L., Tankersley, C. B., Gottlieb, S., Barancik, M., and Sartor, V. E., 1967, Histological demonstration of mucosal invasion in *Giardia lamblia* in man, *Gastroenterology*, 52:143–150.
- Brodsky, P. G., and Wolfe, J. C., 1974, Waterborne giardiasis, *Ann. Intern. Med.* 81:498–499.
- Burke, J. A., 1975, Giardiasis in childhood, *Am. J. Dis. Child.* 129:1304–1310.
- Carroll, M. E., Anast, B. P., and Birch, C. L., 1961, Giardiasis and ureitis, *Arch. Ophthalmol.* 65:775–778.
- Carswell, F., Gobson, A. A. M., and McAllister, 1973, Giardiasis and coeliac disease, *Arch. Dis. Child.* 48:414–418.
- Centers for Disease Control, 1978, Intestinal parasite surveillance, Annual Summary 1977, issued September, 1978, Centers for Disease Control, Atlanta.
- Chandra, R. K., 1980, *Immunology of Nutritional Disorders*,
- Chandra, R. K., and Newberne, P. M., 1977, *Nutrition, Immunity and Infection*, Plenum Press, New York.
- Chandra, R. K., Sahni, S., Dearlove, J., Joglekar, S., Tomas, E., Heresi, G., Antonio, Z., Au, B., Price, B., Brennan, P., Dwyer, J., and Woodford, G., 1982, Clinical and immunological studies of 73 children with selective IgA deficiency, *Afr. J. Clin Exp. Immunol.* 3: submitted for publication.
- Chawla, L. S., Sehgal, A. K., Broor, S. L., Verma, R. S., and Chhuttani, P. N., 1975, Tryptic activity in the duodenal aspirate following a standard test meal in giardiasis, *Scand. J. Gastroenterol.* 10:445–447.
- Conn, M. O., Binder, M., and Burns, B., 1968, Pernicious anemia and immunologic deficiency, *Ann. Intern. Med.* 68:603–612.
- Cortner, J. A., 1959, Giardiasis, a cause of celiac syndrome, *Am. J. Dis. Child.* 98:311–316.
- Cortner, J. A., 1961, Giardiasis in childhood, *Q. Rev. Pediatr.* 16:218–220.
- Cowen, A. E., and Campbell, C. B., 1973, Giardiasis, a cause of vitamin B₁₂ malabsorption, *Am. J. Dig. Dis.* 18:384–390.
- Danciger, M., and Lopez, M., 1975, Number of *Giardia* in the feces of infected children, *Am. J. Trop. Med. Hyg.* 24:237–242.
- DeMuro, P., 1939, Clinical aspects of giardiasis, *Acta Med. Scand.* 99:78–91.

- Dobell, C., 1932, *Antony von Leeuwenhoek and His "Little Animals,"* Harcourt Brace, New York, pp. 223–225.
- Dobell, C., 1940, Vilem Lambl (1824–1895): A portrait and a biographical note, *Parasitology* 32:122–125.
- Duncombe, V. M., Bolin, T. D., Davis, A. E., Cummins, A. G., and Crouch, R. L. 1978, Histopathology in giardiasis—a correlation with diarrhea, *Aust. N.Z. J. Med.* 8:392–396.
- Erlandsen, S. L., and Chase, D. G., 1974, Morphological alterations in the microvillous border of villous epithelial cells produced by intestinal microorganisms, *Am. J. Clin. Nutr.* 27:1277–1286.
- Fantham, H. B., and Porter, A., 1916, The pathogenicity of *Giardia (lamblia) intestinalis* to man and experimental animals, *Br. Med. J.* 2:139–141.
- Faust, E. C., Sarvitz, W., Tobie, J., Odom, U., Peres, C., and Lincicome, D. R., 1939, Comparative efficiency of various techniques for the diagnosis of protozoa and helminths in feces, *J. Parasitol.* 25:241–262.
- Ferguson, A., McClure, J. P., and Townley, R. R., 1976, Intraepithelial lymphocyte counts in small intestinal biopsies from children with diarrhea, *Acta Paediatr. Scand.* 65:541–546.
- Filice, F. P., 1952, Studies on the cytology and life history of a *Giardia* from the laboratory rat, *Univ. Calif. Publ. Zool.* 57:53–146.
- Fossati, C., 1971, Manifestazioi bronocopulmonari in corso di infestazione da *Giardia lamblia*, *Per. lber. Parasitol.* 31:283–298.
- Gazder, A. J., and Banergee, M., 1977, Single dose treatment of giardiasis in children: A comparison of tinidazole and metronidazole, *Curr. Med. Res. Opin.* 5:164–168.
- Goobar, J. P., 1977, Joint symptoms in giardiasis, *Lancet* 1:1010–1011.
- Goritskaya, V. V., and Vrulerskaya, L. A., 1966, Experimental invasion of white rats with *Lambliia intestinalis*, *Med. Parazitol. (Mosk.)* 35:206–208.
- Gupta, R. K., and Mehta, S., 1973, Giardiasis in children: A study of pancreatic functions, *Indian J. Med. Res.* 61:743–748.
- Haiba, M. H., 1954, The pH of the alimentary tract in the normal and *Giardia* infected culture mice, *Parasitology* 44:387–391.
- Haiba, M. H., 1956, Further study on the susceptibility of musines to human giardiasis, *Z. Parasitenkd.* 17:339–345.
- Harris, R. H., and Mitchell, J. H., 1949, Chronic urticaria due to *Giardia lamblia*, *Arch. Dermatol. Syphilol.* 59:587–589.
- Hass, J., and Bucken, E. W., 1967, Zum Krankheitswert der lambliden Infektion, *Dtsch. Med. Wochenschr.* 92:1869–1871.
- Hegner, R. W., 1926, The biology of host–parasite relationship among protozoa living in man, *Q. Rev. Biol.* 1:393–418.
- Hermans, P. E., Huizeng, K. A., Hoffman, H. N., Brown, A. L., and Markowitz, H., 1966, Dysgammaglobulinemia associated with modular lymphoid hyperplasia of the small intestine, *Am. J. Med.* 40:78–89.
- Hoskins, L. C., Winawer, J. J., Broitman, S. A., Gottlieb, L. S., and Zamcheck, N., 1967, Clinical giardiasis and intestinal malabsorption, *Gastroenterology* 53:265–279.
- Hughes, W. S., Cerda, J. J., Holtzapple, P., and Brooks, F. P., 1971, Primary hypogammaglobulinemia and malabsorption, *Ann. Intern. Med.* 74:903–910.
- Hutchison, J. G. P., Johnston, N. M., Plevey, M. V. P., Thangkhiew, I., and Aidney, C., 1975, Clinical trial of mebendazole, a broad spectrum anthelmintic, *Br. Med. J.* 2:309–310.
- Ingram, V. G., Rights, F. L., Khan, H. A., Hashimi, K., and Ansari, K., 1966, Diarrhea in children of West Pakistan: Occurrence of bacterial and parasitic agents, *Am. J. Trop. Med. Hyg.* 15:743–750.
- Jacobowski, W., and Hoff, J. C. (eds.), 1979, Proceedings National Symposium on Waterborne Transmission of Giardiasis, U.S. Environmental Protection Agency, Cincinnati, Sept. 18–20,

- 1978 (EPA 600-19-79-001), Washington, available through NTIS, Springfield, Virginia, 22151.
- Jokipii, A. M., and Jokipii, L., 1977, Prepatency of giardiasis, *Lancet* 1:1095-1097.
- Jokipii, L., and Jokipii, A. M. M., 1978, Comparison of four dosage schedules in the treatment of giardiasis with metronidazole, *Infection* 6:92-94.
- Jones, E. G., and Brown, W. R., 1974, Serum and intestinal fluid immunoglobulins in patients with giardiasis, *Am. J. Dig. Dis.* 19:791-796.
- Kamath, K. R., and Murugasu, R., 1974, A comparative study of four methods for detecting *Giardia lamblia* in children with diarrhea disease and malabsorption, *Gastroenterology* 66:16-21.
- Kavousi, S., 1979, Giardiasis in infancy and childhood: A prospective study of 160 cases with comparison of quinocrine (Atabrine) and metronidazole (Flagyl), *Am. J. Trop. Med. Hyg.* 28:19-23.
- Kessel, J. F., 1929, Experimental giardiasis in kittens and puppies, *J. Parasitol.* 16:99-100.
- Keystone, J. S., Krajden, S., and Warren, M. R., 1978, Person to person transmission of *Giardia lamblia* in day care nurseries, *Can. Med. Assoc. J.* 119:241-248.
- Kidney, W., and Holland, P. D. J., 1967, Giardiasis in children, *J. Irish Med. Assoc.* 60:375-381.
- Kofoid, C. A., 1920, A critical review of the nomenclature of human intestinal flagellates, cercomonas, chilomastix, trichomonas, tetratrichomonas and *Giardia*, *Univ. Calif. Publ. Zool.* 20:145-168.
- Kotcher, E., Mircenda, M., Esquivel, R., Pena-Chararria, A., Donohugh, D. L., Baldizon, C., Acosta, A., and Apuy, J. L., 1966, Intestinal malabsorption and helminthic and protozoan infection of the small intestine, *Gastroenterology* 50:366-371.
- Kunstler, J., 1882, Sur cinq protozoaires parasites nouveaux, *C. R. Soc. Biol. (Paris)* 95:347-349.
- Levi, G. C., deAvila, C. A., and Neto, V. A., 1977, Efficacy of various drugs for treatment of giardiasis. A comparative study, *Am. J. Trop. Med. Hyg.* 26:564-565.
- Lopez, C. E., Dykes, A. C., Juranek, D. D., Sinclair, S. P., Conn, J. M., Christie, R. W., Lippy, E. C., Schultz, M. G., and Mires, M. H., 1980, Waterborne giardiasis: A community wide outbreak of disease and a high rate of asymptomatic infection, *Am. J. Epidemiol.* 112(4):495-507.
- MacDonald, T. T., and Ferguson, A., 1978, Small intestinal epithelial cell kinetics and protozoal infections in mice, *Gastroenterology* 74:496-500.
- Mahalanabis, D., Simpson, T. W., Chakraborty, M. L., Ganguli, C., Bhatlacharjee, A. K., and Mukherjee, K. L., 1979, Malabsorption of water miscible vitamin A in children with giardiasis and ascariasis, *Am. J. Clin. Nutr.* 32:313-318.
- Matthews, C., and Choudry, R. A., 1972, Giardiasis: A preliminary trial of therapy with erythromycin, *Asian J. Med.* 8:193-195.
- Mayer, E. A., and Radulescu, S., 1979, *Giardia* and giardiasis, *Adv. Parasitol.* 17:1-47.
- Mayoral, L. G., Tripathy, K., Garcia, F. T., and Ghitis, J., 1966, Intestinal malabsorption and parasitic disease: The role of protein malnutrition, *Gastroenterology* 50:856-857.
- McClelland, D. B. L., Warwick, R. R. G., and Shearman, D. J. C., 1973, IgA concentration, *Am. J. Dig. Dis.* 18:347-348.
- Meuwissen, J. H. E., Th. Tongeren, J. H. M. V., and Werkman, H. P. T., 1977, Giardiasis, *Lancet* 2:32-33.
- Meyer, E. A., and Jarrell, E. L., 1980, Giardiasis, *Am. J. Epidemiol.* 111:1-12.
- Meyers, J. D., Kuharic, H. A., and Holmes, K. K., 1977, *Giardia lamblia* interaction in homosexual men, *Br. J. Vener. Dis.* 55:54-55.
- Moore, G. T., Cross, W. M., McGuire, D., Mollohan, C. S., Gleason, N. W., Healy, G. R., and Newton, L. H., 1969, Epidemic giardiasis at a ski resort, *N. Engl. J. Med.* 281:402-407.
- Morecki, R., and Parker, J. G., 1967, Ultrastructural studies of the human *Giardia lamblia* and subjacent jejunal mucosa in a subject with steatorrhea, *Gastroenterology* 52:151-164.
- Naik, S. R., Rau, N. R., Vinayak, V. K., Narayan, V. A., Zungurwade, S., Sehgal, S. C., and

- Talwar, P., 1978, Presence of *Candida albicans* in normal and in *Giardia lamblia* infected human jejunum, *Ann. Trop. Med. Parasitol.* 72:493–494.
- Naik, S. R., Kumar, L., Naik, S., Sehgal, S., Rau, N. R., and Vinayak, V. K., 1979, Immunological studies in giardiasis, *Ann. Trop. Med. Parasitol.* 73:291–292.
- Naiman, H. L., Sekla, L., and Albritton, W. L., 1980, Giardiasis and other intestinal parasitic infections in a Manitoba residential school for mentally retarded, *Can. Med. Assoc. J.* 122:185–188.
- Nash, P. H., 1976, Giardiasis, *Can. Med. Assoc. J.* 115:18–19.
- Nauruzbaeva, E. C., 1969, On possible participation of mice in transfer of lamblasis agent, *Vestnik Zool.* 3:90–92.
- Nemanic, P. C., Owen, R. L., Stevens, D. P., and Mueller, J. C., 1979, Ultrastructural observations on giardiasis in a mouse model 11. Endosymbiosis and organelle distribution in *Giardia muris* and *Giardia lamblia*, *J. Infect. Dis.* 140:222–228.
- Owen, R. L., Nemanic, P. C., and Stevens, D. P., 1979, Ultrastructural observations on giardiasis in a murine model, *Gastroenterology* 76(4):757–769.
- Padchenko, I. K., and Stolyarchuk, N. G., 1970, Dogs as a spontaneous carrier of *lamblia* and probably source and vector of lamblasis in nature, *Vestnik Zool.* 4:56–61.
- Palumbo, P. J., Scudamore, H. H., and Thompson, J. H., 1962, Relationship of infestation with *Giardia lamblia* to intestinal malabsorption syndromes, *Proc. Mayo Clin.* 37:589–598.
- Petersen, H., 1972, Giardiasis (lamblasis), *Scand. J. Gastroenterol.* 7(Suppl. 14):1–44.
- Powell, E. D. U., 1956, Giardiasis, *Irish J. Med. Sci.* 34:510–517.
- Pugh, R. J., and Newton, R. W., 1980, Giardiasis in infancy and childhood, *Practitioner* 224:393–397.
- Quinn, R. W., 1971, The epidemiology of intestinal parasites of importance in the United States, *South Med. Bull.* 59:29–30.
- Rendtorff, R. C., 1954, The experimental transmission of human intestinal protozoan parasites, *Am. J. Hyg.* 59:209–220.
- Rendtorff, R. C., and Holt, C. J., 1954, The experimental transmission of human intestinal protozoan parasites, *Am. J. Hyg.* 60:327–338.
- Ridley, M. J., and Ridley, D. S., 1976, Serum antibodies and jejunal histology in giardiasis associated with malabsorption, *J. Clin. Pathol.* 29:30–34.
- Roberts-Thomson, I. C., Stevens, D. P., Mahmoud, A. A. F., and Warren, K. S., 1976, Acquired resistance to infections in an animal model of giardiasis, *J. Immunol.* 117:2036–2037.
- Rosenthal, P., and Liebman, W. M., 1980, Comparative study of stool examination, duodenal aspiration, and pediatric entero test for giardiasis in children, *J. Pediatr.* 96:278–279.
- Rustia, M., and Shubik, P., 1972, Induction of lung tumors and malignant lymphomas in mice by metronidazole, *J. Natl. Cancer Inst.* 48:721–729.
- Saxe, L. H., 1950, Transformation studies on the host specificity of the enteric protozoa of rodents, *J. Parasitol.* 36:21–22.
- Schmerin, M. J., Jones, T. C., and Klein, H., 1978, Giardiasis: Association with homosexuality, *Ann. Intern. Med.* 88:801–803.
- Schmidt, G. D., and Roberts, L. S. (eds.), 1977, Giardiasis, in: *Foundations of Parasitology*, C. V. Mosby, St. Louis, pp. 80–85.
- Schultz, M. G., 1975, Giardiasis, *J.A.M.A.* 233(13):1383–1384.
- Sebesteny, A., 1974, The transmission of intestinal flagellates between mice and rats, *Lab Anim.* 8:79–81.
- Sharapov, M. B., and Solov'er, M. M., 1977, Susceptibility of mice and rats to human *lamblia*. Experimental study with the use of a cultivation method, *Med. Parazitol. (Mosk.)* 46:457–461.
- Sheehy, T. N., and Holley, M. P., 1975, *Giardia* induced malabsorption in pancreatitis, *J.A.M.A.* 233(13):1373–1375.

- Simon, C. E., 1922, A critique of the supposed rodent origin of human giardiasis, *Am. J. Hyg.* 2:406–434.
- Singh, R. M., Gupta, B. D., Gupta, M., and Kochar, S., 1977, A comparative study of funazolidone and metronidazole in giardiasis, *Indian J. Pediatr.* 44:183–198.
- Smith, J. W., and Wolfe, M. S., 1980, Giardiasis, *Annu. Rev. Med.* 31:373–383.
- Stevens, D. P., 1982, Host resistance to *Giardia*, *Rev. Infect. Dis.*, submitted for publication.
- Tandon, B. H., Tandon, R. K., Statpathy, B. K., and Shriniwas, 1977, Mechanisms of malabsorption in giardiasis: A study of bacterial flora and bile salt deconjugation in upper jejunum, *Gut* 18(3):176–181.
- Thompson, A., Rowland, R., Hecker, R., Gibson, G. E., and Reid, D. P., 1977, Immunoglobulin-bearing cells in giardiasis, *J. Clin. Pathol.* 30:292–294.
- Tomkins, A. M., Wright, S. G., Drasar, B. S., and James, W. P. T., 1978, Colonization of jejunum by enterobacteria and malabsorption in patients with giardiasis, *Gut* 17:397.
- Tsuchiya, H., 1931, Changes in morphology of *Giardia lamblia* as affected by diet, *Proc. Soc. Exp. Biol. Med.* 28:708–709.
- Uthman, S. M., and Norton, R. A., 1969, The prevalence and morbidity of giardiasis, *Lahey Clin. Found. Bull.* 18:51–53.
- Veazie, L., 1969, Epidemic giardiasis, *N. Engl. J. Med.* 281(15):853.
- Veghelyi, P. V., 1938, Giardiasis in children, *Am. J. Dis. Child.* 56:1231–1241.
- Veghelyi, P. V., 1939, Celiac disease imitated by giardiasis, *Am. J. Dis. Child.* 57:894–899.
- Vinayak, V. K., Jain, P., and Naik, S. R., 1978, Demonstration of antibodies in giardiasis using the immunodiffusion technique with *Giardia* cysts as antigen, *Ann. Trop. Med. Parasitol.* 72:581–582.
- Voogd, C. E., Van der Stel, J. J., and Jacobs, J. J., 1974, The mutagenic action of nitroimidazole and sonidazok, *Mutat. Res.* 26:483–490.
- Walzer, P. D., Wolfe, M. S., and Schultz, M. G., 1971, Giardiasis in travelers, *J. Infect. Dis.* 124:235–237.
- Weiss, H. B., Winegar, D. A., Levy, B. S., and Washburn, J. W., 1977, Giardiasis in Minnesota, 1971–1975, *Minn. Med.* 60:815–820.
- Welch, P. B., 1943, Giardiasis with unusual clinical findings. Preliminary report, *Am. J. Dig. Dis.* 10:52–55.
- Wolfe, M. S., 1975, Giardiasis, *J.A.M.A.* 233:1362–1365.
- Wolfe, M. S., 1978, Current concepts in parasitology, *N. Engl. J. Med.* 298:319–321.
- Wolfe, M. S., 1979, Giardiasis, *Pediatr. Clin. North Am.* 26:295–303.
- Wright, S. G., and Tomkins, A. M., 1977, Quantification of the lymphocytic infiltrate in jejunal epithelium in giardiasis, *Clin. Exp. Immunol.* 29:408–412.
- Wright, S. G., Tomkins, A. M., and Ridley, D. S., 1977, Giardiasis: Clinical and therapeutic aspects, *Gut* 18(5):343–350.
- Yardley, J. H., Takano, J., and Hendrix, T. R., 1964, Epithelial lesions of the jejunum in giardiasis. Jejunal biopsy studies, *Johns Hopkins Med. J.* 115:389–406.
- Zamcheck, N., Hoskins, L. C., Winawer, J., Broitman, S. A., and Gottlieb, L. S., 1963, Histology and ultrastructure of the parasitic and the intestinal mucosa in human giardiasis. Effects of antabrine therapy, *Gastroenterology* 44:860.
- Zinneman, M. M., and Kaplan, A. P., 1972, The association of giardiasis with reduced intestinal secretory immunoglobulin A, *Am. J. Dig. Dis.* 17:793–797.
- Zisman, M., 1977, Blood-group A and giardiasis, *Lancet* 2:1285.

Immunopathology of Leprosy

J. L. TURK

1. INTRODUCTION

Mycobacterial diseases have been a fruitful field for research into the nature of chronic inflammatory processes since the discovery of mycobacteria as a cause of disease by Robert Koch. Until 20 years ago much of this research has been within the field of tuberculosis. However, within the last 20 years, leprosy has taken its place with tuberculosis as an increasingly popular area, and it could justifiably be said that over this time more has been learned about the immunopathology of chronic infectious diseases from current studies in the field of leprosy than from the study of any other single infection. Leprosy provides a model in which it is possible to study disease caused by delayed hypersensitivity reactions, immune complex disease, and the effects of immunodeficiency as the result of infection with the same organism.

Despite an inability to culture this organism *in vitro*, recent advances in knowledge of immunologic processes in disease have stimulated research in this disease. It is likely that this is because of the ease of studying a predominantly cutaneous disease as compared with tuberculosis where lesions are mainly confined to the internal organs. Progressive changes in the immunologic status of the patient can be learned by superficial observation, and precision obtained by the easy and ethical approach of cutaneous biopsy. In this way, it is possible to build up a complete pattern of disease in a way that would be impossible in other states affecting predominantly the lungs, kidneys, or bones. With the development of modern tests of cell-mediated immunity and tissue immunology coupled with

highly sensitive antibody and complement component assays, it has been possible to build up a pattern of clinico-pathological correlations that will be a model for disease analysis for many years to come. Each disease pattern merging one into the other can be analyzed for the contribution of the different components of the immune system towards its particular manifestations.

Mycobacteria are facultative intracellular parasites. During infection they are found mainly in the body within cells of the mononuclear phagocyte series. Because of their cell wall, they cannot be eliminated from the body by mechanisms dependent on circulating humoral antibody. Macrophages eliminate mycobacteria from the body through cell-mediated immune processes. Chronic infection with mycobacteria results from the infecting organisms developing qualities that protect them from the host's defense mechanisms. Poor host resistance may be as much the result of a low degree of antigenicity as of a weakness in the host's immunologic recognition mechanisms. Mycobacteria appear to play little direct role in the pathological processes underlying the diseases they cause. They do not secrete exotoxins, nor do they carry endotoxins as part of their cell wall. The chronicity of infection with these organisms appears, therefore, to depend as much on the ability of the organism to avoid host defense mechanisms as on its low toxicity which allows it to survive within the host in a prolonged state of parasitism.

Pathological processes in mycobacterial infections are generally the result of hypersensitivity mechanisms in situations in which the immunologic processes underlying these hypersensitivity mechanisms are separate from those underlying host resistance. Dissociation between hypersensitivity and resistance may be because of processes directed against different antigens in the organism or under conditions in which tissue damage may result from antibody-mediated mechanisms and where host resistance may be poor as a result of failure in cell-mediated immunity. During infections with mycobacteria, one may observe proliferation of the organism because of a specific failure of cellular immunity and strong hypersensitivity reactions resulting from the interactions between antigens derived from the infecting organism and high levels of humoral antibody directed against these antigens.

Many acute and chronic infectious diseases are complicated by the superimposition of immune complex disease. The cause of this state is a dissociation between the immune mechanisms underlying host resistance, which is often cell mediated, and the ability to produce complement-activating antibody. Thus, failure of host resistance allows the organism to proliferate. There is a release of soluble antigen, and B lymphocytes are stimulated to produce antibody. Soluble complexes are then formed either in the circulation or locally in the tissues. Complement becomes activated, and inflammation is initiated in particular sites in the body such as the skin, joints, and kidneys.

2. THE IMMUNOLOGIC CLASSIFICATION OF LEPROSY

Current advances in the immunopathology of mycobacterial diseases stem from the introduction of a five-group system by Ridley and Jopling (1962, 1966) for the classification of leprosy according to immunity (Table I). Initially this classification depended heavily on a number of clinical as well as histological criteria, together with the lepromin test and the "bacteriological index." Histologically, the granulomas at the tuberculoid end of the spectrum (TT and BT) are characterized by well-developed "epithelioid cells" with or without Langhans giant cells and are surrounded by a dense zone of lymphocytic infiltration. "Epithelioid cells" are also a feature of borderline leprosy (BB), although the participation of lymphocytes is variable. At the lepromatous pole (BL and LL), the granuloma is composed of "histiocytes" (macrophages) showing varying degrees of fatty change leading to typical foam cells. The cells are highly bacilliferous, and lymphocytes are generally scanty. Some cases of BL leprosy, however, are characterized by a higher proportion of lymphocytes in the granuloma than cases of BB (borderline) leprosy. In a further report (Ridley, 1974), it was decided to subdivide LL into LL_s (subpolar) and LL_p (polar): LL_s granulomas showed less foamy change in the macrophages and more lymphocytes than those from LL_p patients. It was found difficult to classify the lesions of patients without granulomas within this spectrum. In these cases, the lesion was placed within an "indeterminate" group. Patients classified within the spectrum as TT or LL_p are found to have a clinically stable form of the disease. However, those classified histologically between BT and LL_s "upgrade" towards the T end of the spectrum or "downgrade" towards the L end.

It can be seen that there is a progressive increase in lymphocytic infiltration of lesions from the L to the T pole. Conversely, there is a progressive increase in organisms in the skin from the T to the L pole. As will be discussed in more detail later, lepromin skin test positivity is a feature of the T end of the spectrum, and negativity is a feature of the L end. Similarly, patients with tuberculoid disease show a positive lymphocyte transformation test *in vitro* with *Mycobac-*

Table I
Fixed Points on Leprosy Spectrum

TT	Tuberculoid
BT	Borderline tuberculoid
BB	Borderline
BL	Borderline lepromatous
LL _s	Lepromatous (subpolar)
LL _p	Lepromatous (polar)

terium leprae antigens, whereas this reaction is negative in patients with lepromatous disease. Immune complex disease, as might be expected, is only found at the L end of the spectrum, rarely at BL, mostly at LL_s and LL_p.

Mycobacterium leprae may frequently be found in nerves. As a result, nerves may become the site of granuloma formation at the tuberculoid end of the spectrum. In some cases at the T end of the spectrum, the lesions may be predominantly neural. Nerve involvement becomes progressively less towards the L end. Perineural infiltration giving an "onion skin" appearance may, however, be found at BB, BL, and LL_s (Ridley, 1974).

3. THE MONONUCLEAR PHAGOCYTE IN LEPROSY— ULTRASTRUCTURAL STUDIES

Mycobacterium leprae is a facultative intracellular parasite and is found in most granulomas lying within cells of the mononuclear phagocyte series (MPS). It is generally accepted that host resistance in mycobacterial infection depends very strongly on cell-mediated immunity. Cells of the MPS predominate in all leprosy granulomas. At the T end of the spectrum, in granulomas associated with a high state of delayed hypersensitivity, they take on the morphological appearance of epithelioid cells. At the L end of the spectrum, where host resistance is almost nonexistent, these cells are found in the form of macrophages crammed with large numbers of *M. leprae* forming globi.

Ridley *et al.* (1980) made a study of the ultrastructure of leprosy granulomas across the spectrum, paying particular attention to cells of the mononuclear phagocyte system. In BT leprosy the epithelioid cell granulomas were found to contain macrophages with many membrane-bound vesicles (Figures 1, 2). These vesicles had in them material of varying electron density. No obvious bacteria or bacterial debris could be found within these cells. Giant cells of the Langhans type were seen with a similar cytoplasmic picture. In BT leprosy in reaction, however, the cells of the MPS had a different appearance, containing arrays of rough endoplasmic reticulum (Figure 3). In the epithelioid cell granuloma of the Mitsuda skin test (see below), the cells of the MPS contained far larger amounts of endoplasmic reticulum. Between BB and LL_p the cells were fairly typical macrophages in which bacilli were found in varying amounts, depending on the extent of host resistance, within membrane-bound vacuoles. An important question that arises out of this study is "What is an epithelioid cell?"

The epithelioid appearance of cells of the mononuclear phagocyte system is derived from the overall appearance of the cells in these granulomas. Superficially, they appear frequently to be oriented in one direction or another and occasionally organized in whorl-like or "onion skin" pattern surrounding a central area that may contain giant cells or be the site of necrosis as in most cases

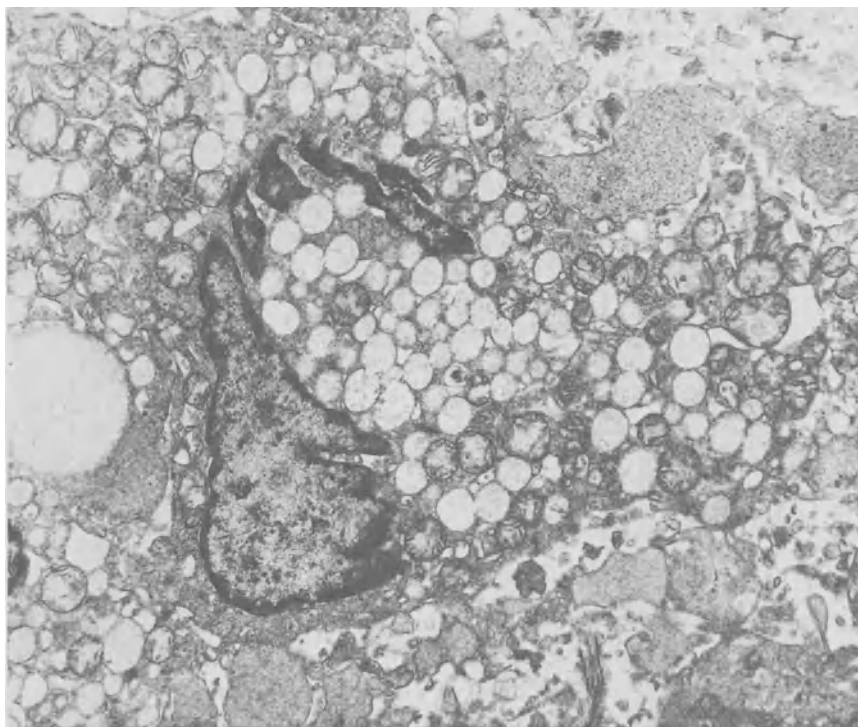


Figure 1. Commonest cell of mononuclear phagocyte series in epithelioid cell granuloma in BT leprosy containing many intracellular vacuoles ($\times 9138$).

of fibrocaceous tuberculosis. Epithelioid cells as such can only be recognized ultrastructurally, using the electron microscope. Two types of cells of the mononuclear phagocyte series may be found in "epithelioid cell granuloma." One type specific to these granulomas has a distinctive appearance. The nucleus is round or oval with finely margined nuclear chromatin. Nucleoli are large, spherical, and reticulated. A typical feature is the large amount of endoplasmic reticulum. Mitochondria are well developed and sometimes swollen. The cytoplasm contains a number of vesicles and vacuoles. Phagocytosis is an occasional feature but does not occur regularly. These cells are secretory cells, and granulomas containing these cells are often associated with a considerable amount of fibroblast proliferation (Turk *et al.*, 1978). The other cell type of the mononuclear phagocyte system found in these granulomas is more like an activated macrophage and has minimal rough endoplasmic reticulum. It is frequently highly vacuolated and contains large numbers of mitochondria and pinocytotic vacuoles and dense bodies. The latter type is the one commonly found in "epithelioid cell granulomas" towards the tuberculoid pole of the leprosy spectrum.

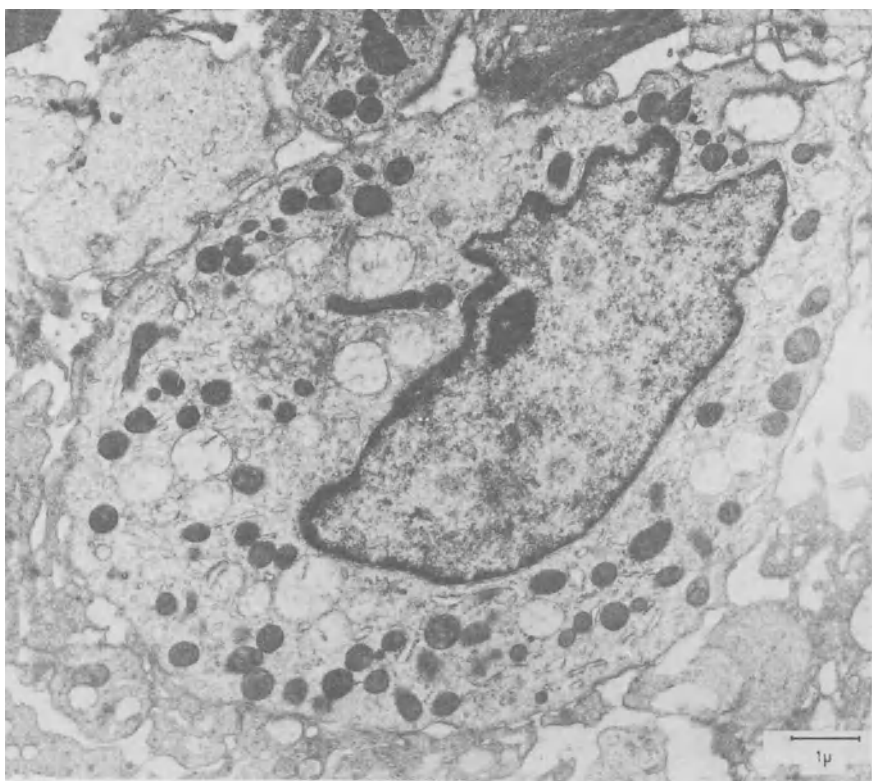


Figure 2. Cell of mononuclear phagocyte series with intracellular vacuoles and some endoplasmic reticulum in epithelioid cell granuloma of BT leprosy ($\times 9000$).

In other mycobacterial granuloma, such as that produced by BCG vaccine in the guinea pig, the "secretory" type of epithelioid cell may be more common.

It appears that the secretory type of epithelioid cell is found mainly in acute epithelioid cell granuloma rather than in those that are long standing. Moreover, these granulomas show marked fibroblast proliferation and collagen deposition. Lymph nodes draining the skin, such as the epitrochlear node, give a good measure of the state of the immune response in man (Turk and Waters, 1971). At the lepromatous end of the spectrum, the paracortical (T-cell) areas are infiltrated with macrophages packed with mycobacteria. As resistance increases across the spectrum, the histiocytes become more differentiated, eventually becoming epithelioid in BT nodes. This is paralleled by increasing numbers of lymphocytes (presumably T-cells) in the paracortical area. In BT nodes the appearance is very similar to that in sarcoidosis lymph nodes. In polar TT there is no infiltration,

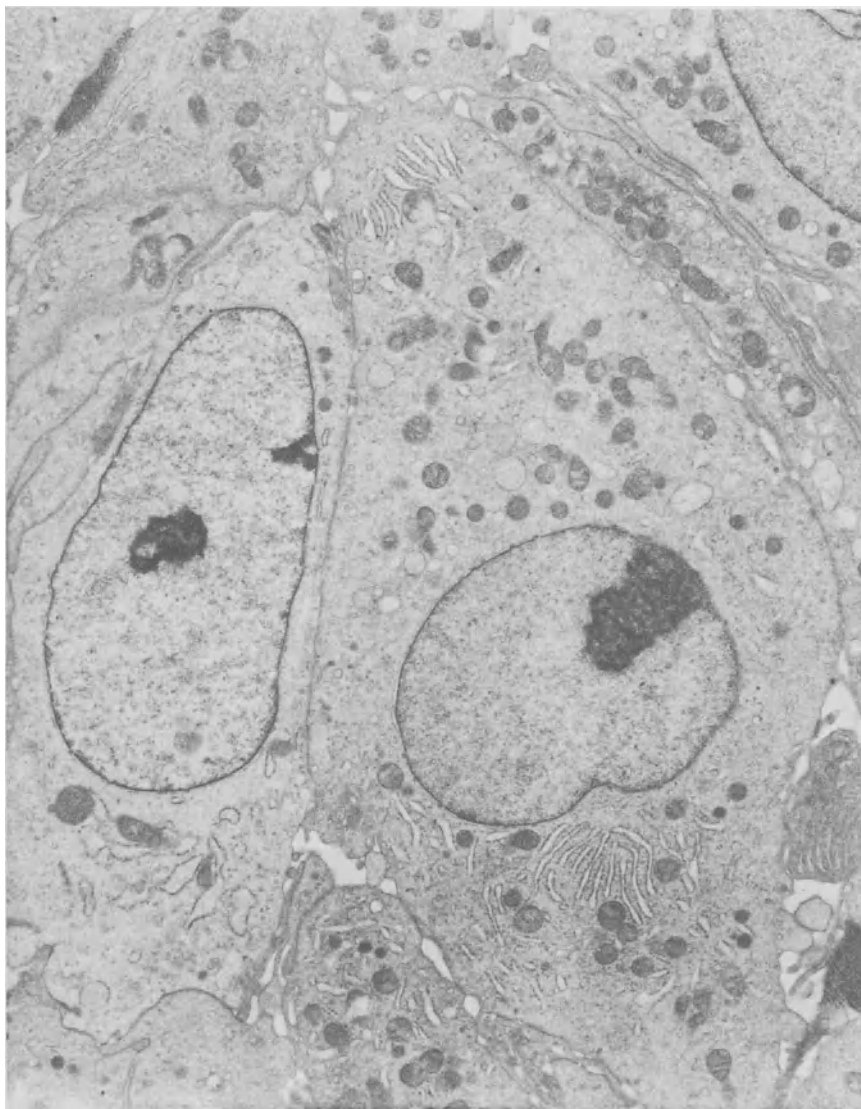


Figure 3. Cell of mononuclear phagocyte series in epithelioid cell granuloma of BT leprosy in reaction, containing arrays of rough endoplasmic reticulum ($\times 6250$).

and the paracortical areas are well developed and populated with lymphocytes and immunoblasts.

In order to study mycobacterial granulomas in more detail, a systematic study was made of cells of the mononuclear phagocyte system in guinea pig lymph nodes. Live BCG induces a granuloma that peaks at 2–3 weeks, and by light microscopy a large number of the infiltrating cells have the appearance of epithelioid cells. Ultrastructurally, these cells are characterized by prominent nucleoli and paucity of cytoplasmic organelles and swollen rough endoplasmic reticulum (Figure 4). These granulomas resolve slowly, and by light and electron microscopy fibroblast proliferation and collagen deposition was seen by 7 weeks and was very extensive in some nodes at 10 weeks. In contrast, the nodes from *M. leprae*-injected animals showed peak infiltration at 5 weeks, and by light microscopy the infiltrating cell population has the appearance of macrophages.

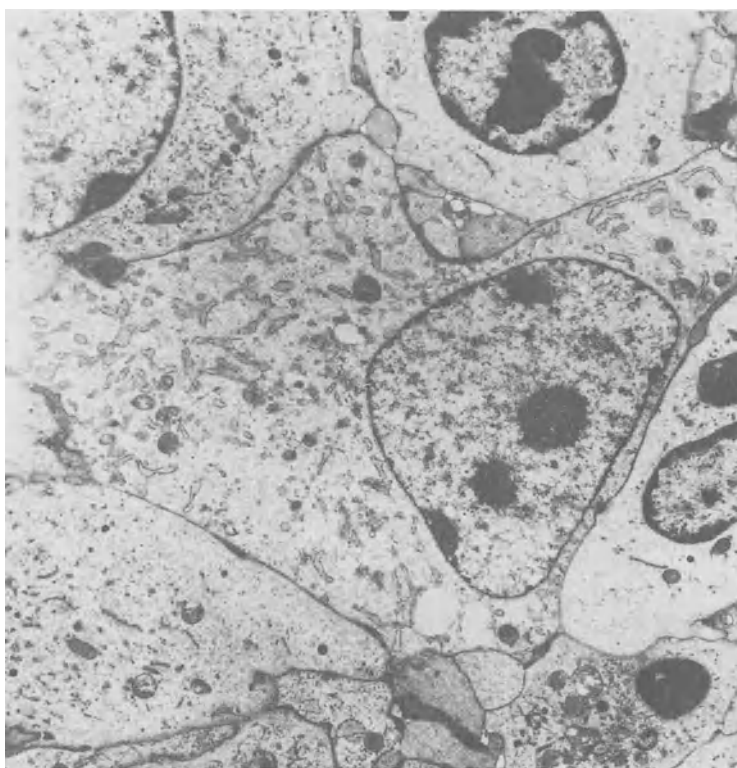


Figure 4. Cell of mononuclear phagocyte series found in epithelioid cell granuloma in draining lymph node 2 weeks after intradermal BCG inoculation, containing arrays of rough endoplasmic reticulum (guinea pig) ($\times 4400$).

Electron microscopy showed these infiltrating cells to be mainly activated macrophages containing phagocytosed organisms. Thus, the lymph nodes draining areas of local injection of BCG and *M. leprae* injected intradermally into the guinea pig ear form, respectively, good models for the study of the granulomas of tuberculoid and lepromatous leprosy in man (Narayanan *et al.*, 1981).

These granulomas can be quantified by measurement of lymph node weight and the areas of infiltration in histological sections. The time course of granuloma formation by *M. leprae* is very different from that induced by BCG. The BCG granuloma develops rapidly and resolves quickly, whereas that induced by *M. leprae* has a slower evolution and resolution. Increased collagen synthesis, as assessed by the incorporation of [^{14}C]proline into collagenase-sensitive tissue, was evident in BCG nodes but remained at basal levels in the *M. leprae* nodes. The more rapid resolution of BCG granulomas could be shown to be related to the stimulation of collagen synthesis in granulomas containing epithelioid cells with rough endoplasmic reticulum (RER). *Mycobacterium leprae* granulomas in which cells of the MPS took on the form of cells with minimal RER showed no increased collagen synthesis and poor resolution.

Granulomas in the tissues in mycobacterial infections may also be differentiated by membrane receptors on cells of the mononuclear phagocyte system. Membrane receptors for the Fc fragment of IgG and C3 can be detected on the surface of peripheral blood monocytes and tissue histiocytes by EA and EAC adhesion. Epithelioid cell granulomas in tuberculoid (BT and TT) leprosy, sarcoidosis, and Buruli ulcer (*M. ulcerans*) show marked adherence of EAC to the area of epithelioid cells in the center of the granuloma in cryostat sections. In lepromatous leprosy, there is adherence of EA but not EAC, and in borderline leprosy (BB and BL) there is adherence of both EA and EAC (Ridley *et al.*, 1978). This indicates a loss of Fc receptors from the membranes of cells of the mononuclear phagocyte series in epithelioid cell granulomas. In contrast, in the nonimmunologic granulomas of lepromatous leprosy, there is a loss of C3 receptors with a preservation of Fc receptors. A similar loss of Fc receptors from mouse macrophages was found when they developed an epithelioid cell appearance after adhering to implanted coverslips in mice (Mariano *et al.*, 1976). Loss of C3 receptors can be induced *in vitro* in macrophages by feeding them BCG vaccine (Ridley *et al.*, 1979).

4. REACTION STATES IN LEPROSY

Ridley (1969) classified reaction states into "downgrading" and "reversal" reactions and erythema nodosum leprosum (ENL). "Downgrading" reactions are associated with a decline in immunity with rapid movement across the spectrum towards the lepromatous end. This is associated with an increase in the

number of bacilli in the tissues and occurs only in patients in the borderline-to-tuberculoid region. Reversal reactions occur in patients in the near-lepromatous and borderline region when the bacterial load is diminished as a result of treatment and is associated with a rapid movement across the spectrum to the BB or BT position. Reversal reactions take the form of erythematous indurated painful lesions in the skin and severe inflammation of the nerves. There is an influx of lymphocytes into the tissues, and the granulomas become epithelioid cell type, often with Langhans-type giant cells. Necrosis is a not infrequent feature. A reversal reaction is caused by the development of severe delayed hypersensitivity lesions in the tissues. Examination of the epitrochlear lymph nodes in patients in this state show the beginning of infiltration of depleted paracortical areas by lymphocytes, particularly around the postcapillary venules.

Erythema nodosum leprosum (ENL) takes the form of typical crops of small painful erythematous nodules, occasionally leading to ulceration and necrosis. The appearance may be very much like that of erythema multiforme and occasionally may take on a hemorrhagic appearance. Erythema nodosum leprosum may be associated with other stigmata of immune complex disease such as iridocyclitis, arthritis, and nephritis. In addition, there may be swelling of the liver, spleen, and lymph nodes. Erythema nodosum leprosum is often associated with painful swollen nerves. Frequently, there is a pyrexia and polymorphonuclear leucocytosis. Histologically, the major feature of these lesions is a polymorphonuclear leucocyte infiltration. Vasculitis or vascular necrosis may be found in the more severe lesions. Occasionally, there is histological evidence of infarction of small areas of the skin.

The appearance of ENL in the skin is very reminiscent of an Arthus reaction. In a number of more severe cases it has been possible to demonstrate immunoglobulin and C3 deposited in the vessel walls as well as in the surrounding tissues. There may be evidence in addition of mycobacterial antigen in these deposits (Wemambu *et al.*, 1969). Although ENL may be associated with some features of lesions in other tissues suggestive of circulating immune complexes, it is likely that the cutaneous lesions develop as a result of the local interaction between soluble mycobacterial antigen and antibody. Increased circulating C3d levels were found in the plasma of 70% of patients with ENL (Bjorvain *et al.*, 1976), indicating extravascular formation of immune complexes. High C1q binding has been found in 80% of lepromatous patients with or without a history of ENL and in 58% of patients with tuberculoid leprosy, indicating that circulating complexes are not necessarily the cause of ENL. It is interesting that the C1q binding property of the serum decreased by half after incubation with *M. leprae* antigens, indicating that these complexes contained mycobacterial antigen.

The possibility that ENL could be associated with activation of the alternative pathway has not been excluded. *Mycobacterium leprae* has been found to be able to activate C3 in the serum of C4-deficient guinea pigs, indicating that this is

possible (Ramanathan *et al.*, 1980). Erythema nodosum leprosum can be suppressed by the drug thalidomide. How this acts is not known. Although thalidomide has no known immunosuppressive or antiinflammatory action in experimental animals, it has been found to be effective in suppressing certain cutaneous conditions in man, such as Actinic reticuloid and discoid lupus erythematosus (C. D. Calnan, personal communication, 1980).

A different type of reaction found in patients with leprosy is the Lucio phenomenon which is described mainly in patients of Mexican extraction in Central and North America. The lesions are mainly necrotizing and occur only in patients with diffuse nonnodular lepromatous leprosy. The lesions evolve into typical hemorrhagic infarcts (Rea, 1979). Histologically, there is ischemic epidermal necrosis and endothelial proliferation of the medium-size vessels of the middermis. Immunofluorescent studies reveal immunoglobulin and complement in the vessel walls. The Raji cell test indicates the presence of circulating immune complexes. The patients also have positive cryoglobulins and latex test. The restriction of this type of reaction to patients with diffuse nonnodular lepromatous leprosy, found mainly in Mexico, has caused the suggestion that patients who show this type of reaction have a "singularly deficient defence mechanism" (Rea, 1979). The reason why they develop these unique lesions is not at all clear.

5. THE LEPRONIN TEST

Lepromin was first used as a skin test reagent by Mitsuda (1919). Originally, lepromin was prepared from highly bacilliferous human skin lesions. The tissue is autoclaved and then ground in 0.5% phenol-saline. Thus, the reagent contained heat-killed whole *M. leprae* and human material. Suspensions of lepromin are generally standardized as containing 1.6×10^8 acid-fast bacilli per milliliter. Other lepromins have been produced in which the ground autoclaved material was extracted with chloroform and ether to remove the human material (Dharmendra, 1942). Modern lepromin is produced from infected armadillo tissue. Lepromin positivity occurs at the tuberculoid pole, and patients with lepromatous leprosy are characteristically lepromin negative.

Skin reactions to lepromin are divided into an early and late reaction (Rees, 1964). The early or Fernandez reaction is a typical delayed hypersensitivity reaction occurring 24–48 hr after injection with erythema and induration. This reaction is found with all preparations of lepromin. The late or Mitsuda reaction is a nodular granuloma that reaches its maximum 3–4 weeks after injection and is best developed after use of the whole Mitsuda material. In severe reactions the nodule may ulcerate. Histologically, the Fernandez reaction is similar to that of the tuberculin reaction, with perivascular mononuclear infiltration, although

there may be some hemorrhage and polymorphonuclear leucocyte infiltration indicating a possible added Arthus component. The Mitsuda reaction, however, is a typical epithelioid cell granuloma with giant cell formation healing by fibrosis. A Mitsuda reaction should be confirmed by biopsy to demonstrate the epithelioid cell nature of the granuloma.

Many purified fractions of lepromin have been prepared. Once the bacilli are disrupted, it becomes more difficult to get a late reaction. Integral bacilli are necessary to produce the typical Mitsuda reaction. Fernandez reactions can be produced by ultrasonicated bacilli and by the protein and nucleoprotein fractions (Dharmendra, 1941, 1942). It is an often repeated observation that the Mitsuda reaction gives the best correlations. It must be remembered, however, that the main value of the lepromin test is to demonstrate a failure of host resistance to *M. leprae*, and it is used as a help in the classification of leprosy patients. Mitsuda reactions are negative in BL, LL_s, and LL_p leprosy. Reactions of 8 mm are found at BT and 4.5 mm at BB.

Lepromin positivity does not necessarily indicate any previous contact with *M. leprae* or other pathogenic mycobacteria (Shepard and Saitz, 1967). M. F. R. Waters (personal communication, 1980) lepromin tested 65 normal Caucasians who had never been exposed to a leprosy-endemic area or received BCG and who were tuberculin negative. Only one gave a delayed hypersensitivity reaction at 72 hr; 38%, however, gave positive Mitsuda reactions at 3 weeks. As the subjects gave negative delayed hypersensitivity at 72 hr, the 3-week reactions suggest primary sensitization by the lepromin itself and that a positive Mitsuda reaction indicates only that the individual is capable of reacting to *M. leprae* with a granulomatous reaction, in contrast to patients with lepromatous disease. The presence of a positive Mitsuda reaction in a patient with indeterminate leprosy would indicate that the disease is more likely to recover spontaneously or to develop into tuberculoid rather than into the lepromatous form.

6. EXPERIMENTAL MODELS OF LEPROSY

6.1. *Mycobacterium leprae*

Until 1960, it was not possible to transmit *M. leprae* to experimental animals. Even now, it cannot be grown *in vitro* with any great success. Shepard (1960) was the first to transmit *M. leprae* successfully in experimental animals. From 10^2 to 10^6 *M. leprae* bacilli, derived mainly from human nasal washings, were inoculated into the footpads of mice. With a dose of 10^5 to 10^6 bacilli, macroscopic granulomas were detected at the inoculation site after 1 to 2 months. With 10^3 organisms granulomas took up to 6 months to develop. After the

development of the lesions, the number of bacilli present never exceeded 10^6 organisms regardless of the number injected. This indicated that further proliferation was controlled by host resistance. Rees and his colleagues (Rees, 1965; Rees *et al.*, 1967) injected *M. leprae* into mice that had been thymectomized, given total-body irradiation of 900 rad, and transfused with syngeneic bone marrow at the age of 6 weeks. This procedure considerably enhanced the infection by overcoming host resistance, so that 10^9 bacilli could be obtained from the injection site. In addition, the skin covering the injected foot developed nodular swellings similar to those seen in lepromatous leprosy. An intravenous injection of 3×10^7 bacilli produced a disseminated form of the disease in which lesions could be found in the nose, ears, and both fore and hind paws. This condition took 19 months to develop. If thymectomized irradiated mice with leprosy granulomas were injected intraperitoneally with lymphocytes, it was possible to produce the equivalent of a reversal reaction in which the areas of skin infiltrated with *M. leprae* granulomas became red and swollen. In addition, the bacilli were seen to become granular and degenerate both in the skin and the Schwann cells of the peripheral nerves. The yield of bacilli dropped 10- to 100-fold (Rees and Weddell, 1968). Mice with lesions resembling histologically those found in the BB or BT points on the spectrum could be seen in normal mice injected with *M. leprae* and followed for 2–3 years. These lesions were typical epithelioid cell granulomas containing a few bacilli and surrounded by loosely packed lymphocytes.

In 1971, Kirschheimer and Storrs introduced a new animal model into leprosy research with the demonstration that infection of the nine-banded armadillo with *M. leprae* could result in the development of advanced disseminated leprosy within 15 months, (Storrs, *et al.*, 1974). Further studies indicated that the disease could be produced in 40% or more of animals injected with the organism. As lepromatous tissue from the armadillo may contain over 100 times more organisms than an equivalent amount of human lepromatous tissue, the armadillo has become an important source of organisms for the production of a vaccine as well as skin-testing reagents. In addition, it provides a natural model for the investigation of the disease.

6.2. *Mycobacterium leprae-murium*

Mycobacterium leprae-murium (MLM) was discovered as a cause of a leprosylike disease in rats in 1903 (Stefansky, 1903). Kawaguchi (1959), using two inbred strains of mice, C57B1/6 and C3H, was able to produce two polar forms of the disease dependent on the relative susceptibilities of the two different strains following subcutaneous inoculation with the organism. In the C3H strain the disease was reported as running a “malignant” course, whereas as in

C57B1/6 mice the disease was more benign. Results were judged by the size of the leproma at the injection site and the survival of the animals over a 40-week period. The disease was lethal in the C3H strain but not in C57B1/6. These findings were confirmed by Closs (1975) using 2.7×10^6 organisms injected into each hind footpad. He considered that the local swelling of the footpad in C57B1 mice was caused by the onset of an immune reaction which caused the mice to resist the infection. In C3H mice, no evidence of an immune reaction could be detected, and the bacilli continued to grow throughout the infection period. Moreover, C57B1 mice inoculated in one footpad and reinfected in the contralateral footpad showed an increased local reaction to the bacilli which failed to multiply at the second site. A similar state of immunity could also be induced by intraperitoneal injection of the organism. Heavy infection with a large dose of *M. leprae-murium* (5×10^8) failed to cause a similar state of immunity because of a state of "immune paralysis."

Closs and Haugen (1974) studied the susceptibility of a number of inbred strains of mice. Among the five inbred strains studied, the strongest and weakest reactions were found in C57B1 and C3H strains, respectively. The capability to respond would, therefore, appear to depend on genetic factors of the host. In none of the strains was host resistance strong enough to stop the proliferation of MLM injected intravenously. However, the lesions in C57B1 mice were surrounded by a marked inflammatory reaction mainly consisting of small lymphocytes, and those in C3H mice showed an aggregate of macrophages without a lymphocytic infiltrate. In a study of the local lymph nodes draining the site of inoculation of MLM, C57B1 mice developed epithelioid cell granulomas similar to those seen in BT leprosy. The C3H mice, however, showed replacement of the paracortical areas by macrophages packed with MLM in a pattern similar to that found in lepromatous (LL_s and LL_p) leprosy (Haugen and Closs, 1975).

Attempts have been made to relate the development of delayed hypersensitivity to host resistance in mice infected with MLM in order to demonstrate a cell-mediated immune basis for host resistance (Poulter and Lefford, 1977, 1978). In C57BL/6 and DBA/2 mice, which were relatively resistant to MLM, the development of delayed hypersensitivity to sonicates of MLM paralleled the initial swelling of the infected foot, the appearance of the mononuclear infiltrate, and prevention of an increase in the number of infecting organisms. A decay in delayed hypersensitivity was associated with increasing proliferation of infecting organisms and was lost altogether following systemic dissemination. Poulter (1978) suggests that specific desensitization and eventually anergy to MLM results from high systemic levels of antigen rather than simply increased numbers of organisms and is encouraged by a too rapid degradation of organisms. In mice infected with 10^8 MLM it could be shown that delayed hypersensitivity was ablated long before resistance to the organism as determined by containment of

bacterial multiplication. Systemic injection of 10^8 live MLM at the same time as local injection was found to suppress the local response to the organism (Lefford and Mackaness, 1977). The way in which such a systemic infection suppresses the immune response may be similar to desensitization, as suggested by Poulter (1978). As lymphocytes from suppressed animals can transfer delayed hypersensitivity passively to normal recipients, the suppressive effect is only active on the efferent limb of the immune response.

BALB/c mice have a similar low resistance to MLM as C3H mice. Alexander and Curtis (1979) compared the delayed hypersensitivity reactions to MLM sonicates in C57BL and BALB/c mice infected with 10^7 MLM. No difference was found in the reactions at 24 hr, but the low-resistance strain BALB/c gave a reaction that peaked 24 hr after skin testing and fell sharply at 48 hr. This reaction was similar to a Jones–Mote type of delayed hypersensitivity. The high-resistance strain C57/BL, however, gave a delayed hypersensitivity reaction that persisted at a high level for 3–4 days, similar to a tuberculin reaction. It was, therefore, suggested that this persistence reaction was a better correlate of host resistance than the 24-hr reaction. Pretreatment of BALB/c mice with cyclophosphamide using a regimen that was known preferentially to deplete suppressor cells regulating the immune response was found to increase the intensity of the delayed hypersensitivity reaction so that it persisted at a high level in the same way as in C57/BL mice. At the same time, it was possible to demonstrate a temporary increase in resistance to the infecting organism (Alexander, 1979).

In addition to the reaction to sonicated MLM, a Mitsuda-type reaction can be produced by injecting mice with an autoclaved preparation of MLM. C57B1 mice give a prolonged footpad reaction that persists for 4 weeks after inoculation. The majority of BALB/c mice gave no increase in footpad thickness. By the use of this lepromin test in addition to other criteria, it was suggested that C57B1 mice fitted into the BT position on the leprosy spectrum, and BALB/c into the BL position (Curtis and Turk, 1979).

An interesting aspect of MLM infection in rats is that infected animals are protected from experimental adjuvant arthritis and from developing a severe form of experimental allergic encephalomyelitis. In addition, skin graft rejection is impaired as well as delayed hypersensitivity reactions to sheep erythrocytes (Bullock *et al.*, 1977). It was suggested that this effect could be caused by an increased production of suppressor cells. Further experiments showed that the early phase of MLM infection was associated with the development of a population of nylon-adherent macrophagelike suppressor cells in the spleen which would depress the plaque-forming cell response of normal spleen cells to sheep erythrocytes. In the later phase of infection, the suppressor cells could be characterized as T lymphocytes by their susceptibility to anti Thy 1.2 serum (Bullock *et al.*, 1978).

7. IN VITRO TESTS OF CELL-MEDIATED IMMUNITY

7.1. Specific Lymphocyte Transformation with *Mycobacterium leprae*

The nature of the immunologic changes that occur in leprosy were studied *in vitro* by Godal and his colleagues (Myrvang *et al.*, 1973) who found that circulating lymphocytes from patients with lepromatous leprosy failed to be transformed *in vitro* by whole *M. leprae* in the lymphocyte transformation test (LTT). There was a similar failure of *M. leprae* to inhibit the migration of leucocytes from capillary tubes. This defect decreased across the leprosy spectrum. Patients at the tuberculoid end showed strong reactivity in both of these tests. Although LL lymphocytes could not be transformed by *M. leprae* in many cases, they could be transformed by BCG.

Initially, it appeared that there was a strong correlation between the specific LTT with *M. leprae* and host resistance. A subsequent report (Bjune *et al.*, 1976) suggested that the correlation was not necessarily with the ability of the host to eliminate the infecting organism but with the strength of the allergic reaction shown by the patient. Thus, LTT may frequently be stronger in actively inflamed BT than in TT. Patients with BT leprosy with silent skin lesions have fairly low LTT responses, whereas those with inflamed lesions have strong responses. Similarly, BL patients with inflamed lesions will react quite strongly in the LTT which may be stronger than that in BT patients in whom there is no evidence of inflammation.

These observations indicate that the LTT response is related to the state of hypersensitivity of the patient rather than to his resistance to infection. Moreover, the response in the lymphocyte transformation test may vary, depending on the nature of the antigenic preparation (Barnetson *et al.*, 1975). Borderline patients with active nerve damage may show a stronger response to sonicated antigen than to whole bacilli. Patients with predominantly cutaneous lesions, however, react better with whole bacilli than with the sonicated preparation.

The nature of the specific defect in lymphocyte responsiveness in lepromatous leprosy has been investigated by Hirschberg (1978). T cells from nonresponding patients were able to respond to *M. leprae* if combined with macrophages from healthy contacts that responded well to *M. leprae*. If combined with macrophages from nonresponding patients, they failed to respond. It would appear from these studies that the basic defect in lepromatous leprosy is a macrophage rather than a T-cell lesion. This also suggests that the Ir gene controlling the immune response to *M. leprae* is to some extent expressed through the macrophage.

However, there is further evidence that suggests that the lymphocyte transformation test is not a reaction with antigens that are primarily associated with

host resistance. Contacts of patients with lepromatous leprosy who do not have any obvious evidence of the disease may still have a low responsiveness to *M. leprae* antigens in the lymphocytic transformation test (Godal and Negassi, 1973).

7.2. Nonspecific Response of Lymphocytes to Mitogens

The first investigation of this type was by Dierks and Shepard in 1968 in which lymphocyte transformation was determined by morphological criteria alone. Lymphocytes from eight active lepromatous patients had a low response to phytohemagglutinin (PHA). In addition, there were three patients with tuberculoid leprosy whose lymphocytes also responded poorly. Sheagren *et al.* (1969) found no differences between the mean of the transformed cells of a control group and groups of leprosy patients using PHA. However, they found reduced responsiveness using Streptolysin O (SLO) as mitogen. Bullock and Fasal (1971) also found depressed transformation with both PHA and SLO. However, reduced transformation to SLO only occurred when autologous plasma was used in the cultures. Lepromatous plasmas would also depress the ability of lymphocytes from normal people to respond to SLO. These plasmas had no effect on PHA transformation. Talwar and his colleagues (1972), using [³H]thymidine uptake, emphasized that peak days of mitotic activity might vary from patient to patient and suggested that comparison should be made of the response on peak days. They also observed that many extrinsic factors including chemotherapeutic treatment might modify the response.

Nelson *et al.* (1971) found differences in the response of lymphocytes to PHA in lepromatous patients of different ethnic groups in Malaysia. Thus, lymphocytes from lepromatous Chinese transformed more strongly than those of normal Chinese whether cultured in autologous or standard normal serum. Cells from lepromatous Malays or Indians transformed less well in autologous serum than in standard normal serum. Serum from Malays or Indians with tuberculoid leprosy depressed the transformation of normal lymphocytes compared with normal serum from individuals of the same ethnic groups. Serum from lepromatous Chinese patients depressed the response of lymphocytes from one normal subject, but not those from another individual. Normal lymphocytes transformed significantly less well when cultured in the serum of lepromatous or tuberculoid Malays or from lepromatous Indians than when cultured in serum from normal controls. No evidence of an intrinsic defect in lymphocyte function could be detected in this study.

It is obvious that these *in vitro* phenomena bear little relation to the pathogenesis of disease. However, they do indicate that data presented from *in vitro* studies of lymphocyte function in isolated ethnic groups should be treated with extreme caution, particularly in attempts to understand the basic failure of host

resistance in lepromatous leprosy. Similar caution should be taken in interpreting later results on the presence of suppressor cells in the circulation of patients with leprosy.

Other reports using red cell rosette techniques and fluorescent antibody evidence of immunoglobulin on the surface of B lymphocytes indicate a decrease in the proportion of T lymphocytes and an increase in the proportion of B lymphocytes in the peripheral blood of lepromatous patients (Dwyer *et al.*, 1973; Gajl-Peczalka *et al.*, 1973; Nath *et al.*, 1974). These changes, including a failure of patients with lepromatous leprosy to be sensitized with DNCB (Waldorf *et al.*, 1966), would appear to develop secondarily to a primary defect in ability to eliminate *M. leprae*. There is no direct parallelism between a number of these defects and the stage of the disease. Moreover, there is no increased susceptibility to infection with other organisms in these patients with depressed lymphocyte function. It is of interest that lepromatous patients who failed to be sensitized with DNCB could still be induced to show delayed hypersensitivity when sensitized with keyhole limpet hemocyanin (Turk and Waters, 1969).

7.3. Suppressor Cells as a Cause of Failure of Cell-Mediated Immunity in Leprosy

The failure to find serum inhibitory factors that might indicate the presence of immunologic enhancement by "blocking" antibodies or immune complexes has led to the current search for the presence of suppressor cells that might account for the failure of host resistance in leprosy. Mehra *et al.* (1979), from the United States, reported that the addition of Dharmendra lepromin to lymphocytes cultured with concanavalin A (Con A) caused a suppression of transformation if the cells were from lepromatous or borderline patients. No such depression was found with the cells of tuberculoid patients or normal individuals. The addition of T cells or an adherent cell population from lepromatous patients was able to suppress the response of lymphocytes from normal individuals to Con A in the presence of lepromin.

In further studies (Mehra *et al.*, 1980), it was found that the cells from lepromatous patients that suppressed the Con A response of normal lymphocytes in the presence of lepromin belonged to a particular subset of T lymphocytes (TH_2^+). These studies were performed using a fluorescent cell sorter and equine anti-human-thymocyte globulin absorbed with T cells from a patient with chronic lymphatic leukemia. The main problem with interpretation of these apparently clear-cut studies is that there are no data available on the ethnic background of the patients used in these studies or on the type of treatment the patients were receiving.

The importance of this becomes clear from other studies performed at the same time but on more defined ethnic groups by Bjune (1979) and Nath *et al.*

(1979). Bjune (1979), working at the Armauer Hansen Research Institute in Addis Ababa on Ethiopian patients, made a similar study stimulating lymphocytes from leprosy patients simultaneously with *M. leprae* antigens and PHA. In all patients across the spectrum from TT to LL_s, sonicated leprosy bacilli and PHA together gave a lower [³H]thymidine incorporation than PHA alone. The effect of sonicated *M. leprae* was dose dependent; BCG was weakly suppressive in combination with PHA, but sonicated *M. duvalii* had a very marked suppressive effect.

A third protocol for the demonstration of suppressor cell activity in peripheral blood of leprosy patients was used by Nath *et al.* (1979) in two groups of patients in India. One group was from New Delhi, the other group from a strong leprosy-endemic area in Wardha. Twenty-four-hour Con A treatment induced suppressor cell activity in tuberculoid leprosy patients that would strongly suppress their own Con A response. The suppressor response in normal and lepromatous patients was very much less. This study would appear to indicate that suppressor cells are generated in proportion to the ability of lymphocytes to respond to mitogen and are not necessarily the cause of a depression in mitogen responsiveness.

Nath and Singh (1980), using autoclaved whole *M. leprae*, found that this would suppress Con A-stimulated lymphocyte transformation in 80% of tuberculoid patients and 35% of untreated lepromatous patients. Healthy contacts and 53% of lepromatous patients showed enhanced Con A responses in the presence of *M. leprae*. The maximum effect on tuberculoid patients was found on the fourth day of culture. On prolongation of antigen presence to 6 days, only marginal effects were found in tuberculoid cultures, whereas the healthy contacts, as well as some lepromatous patients, showed increased inhibition of Con A responses. The presence of *M. leprae* in the cultures inhibited the PPD response of lymphocytes from both leprosy patients and healthy contacts.

In a subsequent study, Nath *et al.* (1980) used HLA-D-identical lymphocyte-lymphocyte and lymphocyte-macrophage cocultures to study these naturally occurring suppressor mechanisms which develop during infection with *M. leprae*. They confirmed their earlier observations that suppressor cells were present in the peripheral blood of tuberculoid patients that would suppress both mitogen- and antigen-induced proliferative responses. They also confirmed the observation of Hirschberg (1978) using HLA-D-identical cells that macrophages from lepromatous patients inhibit lymphoproliferative responses to *M. leprae* antigens. An additional observation by Singh and Nath (1980) showed a decrease in T lymphocytes in lepromatous patients as compared with normal levels of these cells in tuberculoid patients. These findings are in keeping with a decrease in suppressor cell activity in lepromatous leprosy.

It is difficult to reconcile these different observations, and one is reminded of the confusion caused by the different findings on PHA transformation of

lymphocytes across the leprosy spectrum 10 years previously which was partially resolved by the observations of Nelson *et al.* (1971) who showed different responses in patients from different ethnic groups. Similar differences in ethnic groups can be shown in the incidence of autoantibodies in leprosy (Rea *et al.*, 1976). During normal immunization in experimental animals, it can be shown that suppressor cells frequently develop in parallel with a strong immune response (Scheper *et al.*, 1977). The role of these cells is to regulate the stronger immune responses. Thus, it is not surprising that where there is a strong cellular immune response in tuberculoid patients there is a strong negative feedback control mechanism, and where the cellular immune response is weak, as in lepromatous leprosy, the immunoregulatory response is weak. However, this does not explain the strong regulatory cell response demonstrated by Mehra *et al.* (1979, 1980), using a different antigenic system. In view of the suggestion of Hirschberg (1978) and Nath *et al.* (1980) of a defect in macrophage processing of antigen as a possible mechanism underlying the defect in host resistance in lepromatous leprosy, the role of suppressor cells in the pathogenesis of lepromatous disease remains highly controversial.

In assessing the relevance of suppressor cells demonstrated by *in vitro* techniques, it should be emphasized that so far, *in vitro* tests of cell-mediated immunity appear to reflect the allergenic potential of the patient rather than host resistance. Thus, suppressor cells may be more important in controlling and preventing reversal reactions at the tuberculoid end of the spectrum than in playing a role in the failure of host resistance at the lepromatous pole.

8. ANTIBODY RESPONSE IN LEPROSY

High levels of IgG are consistently found in the sera of untreated lepromatous patients. Increased concentrations of IgA and IgM have also been described but appear to be more variable. Antibodies against a number of mycobacterial antigens are found in the sera of patients across the clinical spectrum. However, towards the lepromatous pole there is considerable evidence of an increase in the frequency of these antibodies. Moreover, antibody activity towards mycobacterial antigen is distinctly higher in lepromatous patients.

Mycobacterium leprae is poorly antigenic compared with other mycobacteria. Immunization of rabbits with *M. leprae* grown in armadillos results in antibodies against only seven antigens; this may be compared with BCG which stimulates production of antibodies against 70 components and *M. leprae-murium* which produces antibodies against 30 to 40 antigens. If rabbit serum is concentrated, antibodies may be detected against 20 *M. leprae* antigens. All seven components in *M. leprae* that are reasonably strong antigens cross react with antigens of other mycobacteria, especially BCG. Antibodies against *M.*

leprae antigen 7, which is equivalent to BCG antigen 60, are found in the sera of tuberculoid as well as lepromatous patients (Harboe *et al.*, 1977). Current studies use highly sensitive and specific radioimmunoassays: previous studies have used precipitin assays and have not had the same degree of precision. Antibodies against *M. leprae* antigen 7 are also found, by a similar radioimmunoassay, in the sera of armadillos infected with *M. leprae* (Harboe *et al.*, 1978). Although patients with leprosy usually have antibodies in their serum to no more than four or five antigens found in BCG, it is interesting that one patient has been described with antibodies to 12 BCG antigens (Harboe *et al.*, 1979).

Autoantibodies against a wide range of antigens have been described in patients with lepromatous leprosy (Wager, 1969). These include anti-Ig antibodies (cryoglobulins, rheumatoid factor), antinuclear factor, and antithyroid antibodies. However, the incidence of autoantibodies in these patients appears to vary geographically. Rea *et al.* (1976) found no increased incidence between test and control groups in Southern California. A similar absence of autoantibodies has been found in leprosy patients in Malaysia (M. F. R. Waters, personal communication).

9. GENETIC SUSCEPTIBILITY

It has been postulated that leprosy develops in individuals who had a natural weakness to develop cell-mediated immunity to mycobacterial antigens (Jamison and Vollum, 1968). This hypothesis was put forward as a result of a study in which tuberculin-negative children with a family history of leprosy were vaccinated with vole tuberculosis vaccine. It was found that only 18% of the children converted to tuberculin positivity, as opposed to a 90% conversion rate in children from families without leprosy. Further evidence for genetic control of susceptibility to *Mycobacterium leprae* is obtained from a study of twins with leprosy (Chakravarti and Vogel, 1973) which showed that of 62 monozygotic pairs, 37 (59.9%) were concordant for leprosy, and, of these, 32 were also concordant for the type of leprosy (86.5%). Differences in the intensity of contact with infectious cases and differences in the general state of health were the most likely cause of only one twin being affected in the other pairs. More recently, there has been much interest in the association between leprosy and HLA type. The best study so far has been from the Leiden group (de Vries *et al.*, 1976). Initial studies in Surinam were with HLA-A and HLA-B types. Siblings with the same type of leprosy were found to have a significant excess of identical HLA haplotypes, whereas siblings affected with different types of leprosy shared a haplotype less often than expected. This was taken to indicate that susceptibility to leprosy and the type of disease are controlled by at least two HLA-controlled genes. More recent studies in Wardha (India) by the same group

suggest the association of a particular HLA determinant (DW2) with susceptibility genes for tuberculoid leprosy. In a small study in Ethiopia of HLA-D identity using mixed lymphocyte culture, Stoner *et al.* (1978) compared seven patients with lepromatous leprosy with nine noninfected siblings. Four of the nine were HLA-D identical with their lepromatous siblings, suggesting that susceptibility and the development of lepromatous disease are not under HLA-D control.

10. AMYLOIDOSIS

The association of amyloidosis with leprosy is firmly established. The frequency varies from country to country: 31% in the United States, 7.5% in India, 6% in Mexico, and 2.4% in Malawi. In Papua–New Guinea, the incidence diagnosed by rectal biopsy was 20% in patients with LL_p (McAdam *et al.*, 1975). These patients had a history of either recurrent ENL or chronic ulcers. Levels of the serum protein antigenically related to amyloid (SAA) were found to rise during ENL reactions in parallel with the rise in the polymorphonuclear leucocyte count. The incidence of raised SAA was higher in LL than in other parts of the spectrum (Kronvall *et al.*, 1975). Amyloidosis frequently leads to a nephrotic syndrome which is eventually fatal.

11. IMMUNOPROPHYLAXIS IN LEPROSY

Two approaches have been made towards the use of vaccine against leprosy. The first was to take advantage of the marked antigenic cross reactivity between BCG vaccine and *M. leprae*. The second has been to use cobalt-irradiated *M. leprae* grown in the armadillo. Three major controlled studies have been made of the use of BCG vaccine. These were in Uganda (Brown *et al.*, 1968), Burma (Bechelli *et al.*, 1973), and Karimui, Papua–New Guinea (Russell *et al.*, 1968). In Uganda, 80% Protection was achieved, whereas the protection in Karimui was only 30%, and significant protection in Burma was limited to one small subgroup. In both Karimui and Burma, at the first follow-up examinations 1 year after vaccination, more new cases (indeterminate and tuberculoid) had occurred in the BCG-treated groups than in the control group, but this was not confirmed on subsequent examination. Stanford (1977) has compared the protection conferred by BCG against leprosy in Uganda and Burma with the protection against tuberculosis in the United Kingdom and United States. He considered that the differences observed could have resulted from differences in mycobacteria in the environment and looked at protection against leprosy in Burma and

Uganda as a function of the age at which a child had been vaccinated. If the child was vaccinated before the age of 4, protection was 66% in Burma and 78% in Uganda. If after the age of 4, 25% in Burma and 77% in Uganda. He considered that something had happened to the children in Burma after the age of 4 to change their response to BCG vaccination and suggested that infection with *Mycobacterium marianum* which was more common in Burma, blocked the ability of the older children to respond to BCG.

The WHO Immunology of Leprosy Programme (IMMLEP) has concentrated more on a vaccine using cobalt-irradiated dead *Mycobacterium leprae* grown in the armadillo, as the production of an attenuated live *M. leprae* vaccine comparable to BCG could take many years. Initial problems of isolating *M. leprae* from armadillo tissue using collagenase, trypsin, and chymotrypsin, which tended to destroy protein antigenicity, have now been overcome, and the dead *M. leprae* vaccine has been found to inhibit the limited growth of *M. leprae* in the mouse footpad (Shepard *et al.*, 1978). There is, however, still considerable discussion as to whether a killed vaccine will be effective in increasing host resistance to *M. leprae* in man. The use of an irradiated vaccine may turn out to be the answer to this problem. In view of the problems encountered with the BCG vaccine, it may be many years before a suitable *M. leprae* vaccine has been sufficiently assessed in field trials.

12. SUMMARY

Leprosy may be considered as a model immunologic disease in which to study the role of immunodeficiency, diffuse delayed hypersensitivity reactions in the skin and peripheral nerves, and immune complex disease in the development of the pathological lesions that occur in chronic infectious diseases. Infection with *M. leprae* may induce a wide range of granulomatous lesions depending on the immunologic status of the patient. A dissociation exists between host resistance to the infecting organism and the ability to manifest a hypersensitivity reaction, whether T-cell mediated or produced by the deposition of immune complexes. This results in a broad spectrum of clinical manifestations ranging from the single-lesion, self-healing tuberculoid pole to a specific state of T-cell deficiency with immune complex disease that underlies erythema nodosum leprosum at the lepromatous pole. Much work has been performed in the past decade using currently available *in vitro* techniques for testing the immune status of patients across the leprosy spectrum. In addition, a considerable amount of work is being performed using animal models of mycobacterial disease. The development of an antileprosy vaccine is still some way ahead. However, work along this line sponsored by the WHO shows some promise.

REFERENCES

- Alexander, J., 1979, Effect of cyclophosphamide treatment on the course of *Mycobacterium lepraemurium* infection and development of delayed-type hypersensitivity reactions in C57B1 and BABL/c mice, *Clin. Exp. Immunol.* 34:52–58.
- Alexander, J., and Curtis, J., 1979, Development of delayed hypersensitivity responses to *Mycobacterium lepraemurium* infections in resistant and susceptible strains of mice, *Immunology* 36:563–567.
- Barnetson, R. St. C., Bjune, G., Pearson, J. M. H., and Kronvall, G., 1975, Antigenic heterogeneity in patients with reactions in borderline leprosy, *Br. Med. J.* 4:435–437.
- Bechelli, L. M., Garbajosa, P. G., Gyi, M. M., Uemura, K., Sundarasan, T., Dominguez, V. M., Matejka, M., Tamondon, C., Quagliato, R., Engler, V., and Altmann, M., 1973, BCG vaccination of children against leprosy: Seven year findings of the controlled WHO trial in Burma, *Bull. WHO* 48:323–334.
- Bjorvain, B., Barnetson, R. S., Kronvall, G. K., Zubler, R. H., and Lambert, P. H., 1976, Immune complexes and complement hypercatabolism in patients with leprosy, *Clin. Exp. Immunol.* 26:388–396.
- Bjune, G., 1979, *In vitro* lymphocyte stimulation in leprosy; simultaneous stimulation with *Mycobacterium leprae* antigens and phytohaemagglutinin, *Clin. Exp. Immunol.* 36:479–487.
- Bjune, G., Barnetson, R. S., Ridley, D. S., and Kronvall, G., 1976, Lymphocyte transformation test in leprosy: Correlation of the response with inflammation of lesions, *Clin. Exp. Immunol.* 25:85–94.
- Brown, J. A. K., Stone, M. M., and Sutherland, I., 1968, BCG vaccination of children against leprosy in Uganda: Results at end of second follow up. *Br. Med. J.* 1:24–27.
- Bullock, W. E., and Fasal, P., 1971, Studies of immune mechanisms in leprosy, *J. Immunol.* 106:888–899.
- Bullock, W. E., Evans, P. E., and Filomeno, A. R., 1977, Impairment of cell-mediated immune responses by infection with *Mycobacterium lepraemurium*, *Infect. Immun.* 18:157–164.
- Bullock, W. E., Carlson, E. M., and Gershon, R. K., 1978, The evolution of immunosuppressive populations in experimental mycobacterial infections, *J. Immunol.* 120:1709–1916.
- Chakravarti, M. R., and Vogel, F., 1973, A twin study in leprosy, *Top. Hum. Genet.* 1:1.
- Closs, O., 1975, Experimental murine leprosy: Growth of *Mycobacterium lepraemurium* in C³H and C57/BL mice after footpad inoculation, *Infect. Immun.* 12:480–489.
- Closs, O., and Haugen, O. A., 1974, Experimental murine leprosy 2. Further evidence for varying susceptibility of outbred mice and evaluation of the response of 5 inbred strains to infection with *Mycobacterium lepraemurium*, *Acta Pathol. Microbiol. Scand.* [A] 82:459–474.
- Curtis, J., and Turk, J. L., 1979, Mitsuda type lepromin reactions as a measure of host resistance in *Mycobacterium lepraemurium* infection, *Infect. Immun.* 24:492–500.
- de Vries, R. R. P., Fai, R. F. M. L. A., Nijenhuis, L. E., and van Rood, J. J., 1976, HLA-linked genetic control of host response to *Mycobacterium leprae*, *Lancet* 2:1328.
- Dharmendra, 1941, Studies on the lepromin test (5). The active principle of lepromin is a protein antigen of the bacillus, *Leprosy Ind.* 13:89.
- Dharmendra, 1942, Studies of the lepromin test (9): A bacillary antigen standardized by weight, *Leprosy Ind.* 14:122.
- Dierks, R. E., and Shepard, C. C., 1968, Effect of phytohaemagglutinin and various mycobacterial antigens on lymphocyte cultures from leprosy patients, *Proc. Soc. Exp. Biol. Med.* 127:391–395.
- Dwyer, J. M., Bullock, W. E., and Fields, J. P., 1973, Disturbance of the blood: B lymphocyte ratio in lepromatous leprosy, *N. Engl. J. Med.* 288:1036–1039.

- Gajl-Peczalska, K. J., Lim, S. D., Jacobson, R. R., and Good, R. A., 1973, B lymphocytes in lepromatous leprosy, *N. Engl. J. Med.* 28:1033–1035.
- Godal, T., and Negassi, K., 1973, Subclinical infection in leprosy, *Br. Med. J.* 3:557–559.
- Harboe, M., Closs, O., Bjorvatn, B., and Bjune, G., 1977, Antibodies against BCG antigen 60 in mycobacterial infection, *Br. Med. J.* 2:430–433.
- Harboe, M., Closs, O., Rees, R. J. W., and Walsh, G. P., 1978, Formation of antibody against *Mycobacterium leprae* antigen 7 in armadillos, *J. Med. Microbiol.* 11:525–535.
- Harboe, M., Mshana, R. N., Closs, O., Kronvall, G., and Axelsen, N. H., 1979, Cross-reactions between mycobacteria. 11. Crossed immunoelectrophoretic analysis of soluble antigens of BCG and comparison with other mycobacteria, *Scand. J. Immunol.* 9:115–124.
- Haugen, O. A., and Closs, O., 1975, Experimental murine leprosy 6. Cellular reactions in the draining lymph nodes after injection of *Mycobacterium lepraemurium* into the footpads of mice. *Acta Pathol. Microbiol. Scand.* [A] 83:683–692.
- Hirschberg, H., 1978, The role of macrophages in the lymphoproliferative response to *Mycobacterium leprae* *in vitro*, *Clin. Exp. Immunol.* 34:46–51.
- Jamison, D. G. and Vollum, R. L., 1968, Tuberculin conversion in leprosy families in Northern Nigeria, *Lancet*, 2:1271–1272.
- Kawaguchi, Y., 1959, Classification of mouse leprosy *Jpn. J. Exp. Med.* 29:651–663.
- Kirschheimer, W. F., and Storrs, E. E., 1971, Attempts to establish the armadillo (*Dasypus novemcinctus* Linn.) as a model for the study of leprosy, *Int. J. Leprosy* 39:693.
- Kronvall, G., Husby, G., Samuel, D., Bjune, G., and Wheate, H., 1975, Amyloid-related serum component (protein ASC) in leprosy patients, *Infect. Immun.* 11:969–972.
- Lefford, M. J. and Mackaness, G., 1977, Suppression of immunity to *Mycobacterium lepraemurium* infection, *Infect. Immun.* 18:363–369.
- Mariano, M., Nikitin, T., and Malucelli, B. E., 1976, Immunological and non-immunological phagocytosis by inflammatory macrophages, epithelioid cells and macrophage polykaryons from foreign body granulomata, *J. Pathol.* 120:151–159.
- McAdam, K. P. W. J., Anders, R. F., Smith, S. R., Russell, D. A., and Price, M. A., 1975, Association of amyloidosis with erythema nodosum leprosum reactions and recurrent neutrophil leucocytosis in leprosy, *Lancet* 2:572–575.
- Mehra, V., Mason, L. H., Fields, J. P., and Bloom, B. R., 1979, Lepromin induced suppressor cells in patients with leprosy, *J. Immunol.* 123:1813–1817.
- Mehra, V., Mason, L. H., Rothman, W., Reinherz, E., Schlossman, S. F., and Bloom, B. R., 1980, Delineation of a human T-cell subset responsible for lepromin-induced suppression in leprosy patients, *J. Immunol.* 125:1183–1188.
- Mitsuda, K., 1919, On the value of a skin reaction to suspension of leprosy nodules, *Jpn. J. Dermatol. Urol.* 19:697; reprinted in English, 1953, *Int. J. Leprosy* 21:347–358.
- Myrvang, B., Godal, T., Ridley, D. S., Froland, S. S., and Song, Y. K., 1973, Immune responsiveness to *Mycobacterium leprae* and other mycobacterial spectrum of leprosy, *Clin. Exp. Immunol.* 14:541–553.
- Narayanan, R. B., Badenoch-Jones, P., and Turk, J. L., 1981, Experimental mycobacterial granulomas in guinea pig lymph nodes: Ultrastructural observations, *J. Pathol.* 134:253–265.
- Nath, I., and Singh, R., 1980, The suppressive effect of *M. leprae* on the *in vitro* proliferative responses of lymphocytes from patients with leprosy, *Clin. Exp. Immunol.* 41:406–414.
- Nath, I., Curtis, J., Chutani, L. K., and Talwar, G. P., 1974, Reduction of a subpopulation of T-lymphocytes in lepromatous leprosy, *Clin. Exp. Immunol.* 18:81–87.
- Nath, I., Narayanan, R. B., Mehra, N. K., Sharma, A. K., and Gupte, M. D., 1979, Concanavalin A induced suppressor activity in human leprosy, *J. Clin. Lab. Immunol.* 2:319–324.
- Nath, I., Van Rood, J. J., Mehra, N. K., and Vaidya, M. C., 1980, Natural suppressor cells in

- human leprosy. The role of HLA-D identical peripheral lymphocytes and macrophages in the *in vitro* modulation of lympho-proliferative responses, *Clin. Exp. Immunol.* 42:203-210.
- Nelson, D. S., Nelson, M., Thurston, J. M., Waters, M. F. R., and Pearson, J. M. H., 1971, Phytohaemagglutinin-induced lymphocyte transformation in leprosy, *Clin. Exp. Immunol.* 9:33-43.
- Poulter, L. W., 1978, Systemic immunological reactivity in the absence of delayed-type hypersensitivity during murine leprosy, *Cell. Immunol.* 40:117-127.
- Poulter, L. W., and Lefford, M. J., 1977, Development of delayed-type hypersensitivity during *Mycobacterium lepraemurium* infection in mice, *Infect. Immun.* 17:439-446.
- Poulter, L. W., and Lefford, M. J., 1978, Relationship between delayed-type hypersensitivity and the progression of *Mycobacterium lepraemurium* infection, *Infect. Immun.* 20:530-540.
- Ramanathan, V. D., Curtis, J., and Turk, J. L., 1980, Activation of the alternative pathway of complement by mycobacterium and cord factor, *Infect. Immun.* 29:30-35.
- Rea, T. H., 1979, Lucio's phenomenon: An overview, *Lepr. Rev.* 50:107-112.
- Rea, T. H., Quismorio, F. P., Harding, B., Nies, K. M., Di Saia, P. J., Levan, N. E., and Friou, G. J., 1976, Immunologic responses in patients with lepromatous leprosy, *Arch. Dermatol.* 112:791-800.
- Rees, R. J. W., 1964, The significance of the lepromin reaction in man, *Prog. Allergy* 8:224.
- Rees, R. J. W., 1965, Enhanced susceptibility of thymectomised and irradiated mice to infection with *Mycobacterium leprae*, *Nature* 211:657-658.
- Rees, R. J. W., and Weddell, A. G. M., 1968, Experimental models for studying leprosy, *Ann. N.Y. Acad. Sci.* 154:214-236.
- Rees, R. J. W., Waters, M. F. R., Weddell, A. G. M., and Palmer, L., 1967, Experimental lepromatous leprosy, *Nature* 215:599.
- Ridley, D. S., 1969, Reactions in leprosy, *Lepr. Rev.* 40:77-81.
- Ridley, D. S., 1974, Histological classification and the immunological spectrum of leprosy, *Bull. WHO* 51:451-465.
- Ridley, D. S., and Jopling, W. H., 1962, A classification of leprosy for research purposes, *Lepr. Rev.* 33:119-128.
- Ridley, D. S., and Jopling, W. H., 1966, Classification of leprosy according to immunity. A five group system, *Int. J. Lepr.* 34:255-273.
- Ridley, M. J., Ridley, D. S., and Turk, J. L., 1978, Surface markers on lymphocytes and cells of the mononuclear phagocyte system in skin sections in leprosy, *J. Pathol.* 125:91-98.
- Ridley, M. J., Turk, J. L., and Badenoch-Jones, P., 1979, Modification *in vitro* of membrane receptors on cells of the mononuclear phagocyte system, *J. Pathol.* 127:173-184.
- Ridley, M. J., Badenoch-Jones, P., and Turk, J. L., 1980, Ultrastructure of cells of the mononuclear phagocyte series across the leprosy spectrum, *J. Pathol.* 130:223-227.
- Russell, D. A., Scott, G. C., and Wigley, S. C., 1968, BCG and prophylaxis, the Karimui trial, *Int. J. Lepr.* 36:618.
- Scheper, R. J., Parker, D., Noble, B., and Turk, J. L., 1977, The relation of immune depression and B-cell stimulation during the development of delayed hypersensitivity to soluble antigens, *Immunology* 32:265-272.
- Sheagren, J. N., Block, J. B., Trautman, J. R., and Wolff, S. M., 1969, Immunologic reactivity in patients with leprosy, *Ann. Intern. Med.* 70:295-302.
- Shepard, C. C., 1960, The experimental disease that follows the injection of human leprosy bacilli into foot-pads of mice, *J. Exp. Med.* 112:445-454.
- Shepard, C. C. and Saitz, E. W. 1967, Lepromin and tuberculin reactivity in adults not exposed to leprosy, *J. Immunol.* 99, 637-642.
- Shepard, C. C., Walker, L. L., and Van Ledingham, R., 1978, Heat stability of *Mycobacterium leprae* immunogenicity, *Infect. Immun.* 22:87-93.

- Singh, S., and Nath, I., 1980, Reduction of a subset of T-cells bearing Fc receptors for IgG in lepromatous leprosy, *Int. Arch. Allergy* 62:81–85.
- Stanford, J. L., 1977, A change in attitude towards a vaccine for leprosy and an account of some experiments in animals, in: *Abstracts of a Symposium on Recent Progress in Immunology of Leprosy and Chronic Mycobacterial Infections, New Delhi, November 1977*, All-India Institute of Medical Sciences, New Delhi.
- Stefansky, W. K., 1903, Eine lepraähnliche Erkrankung der Haut und der Lymphdrüsen bei Wanderratten, *Zentralbl. Bakteriol. [Orig. B]* 33:481–487.
- Stoner, G. L., Touw, J., Belehu, A., and Naafs, B., 1978, *In vitro* lymphoproliferative response to *Mycobacterium leprae* of HLA-D-identical siblings of lepromatous leprosy patients, *Lancet* 2:543–547.
- Storrs, E. E., Walsh, G. P., Burchfield, H. P., and Binford, C. H., 1974, Leprosy in the armadillo: New model for biomedical research, *Science* 183:851–852.
- Talwar, G. P., Krishnan, A. D., Mehra, V. L., Blum, E. A., and Pearson, J. M. H., 1972, Evaluation of cell-mediated immune responses in untreated cases of leprosy, *Clin. Exp. Immunol.* 12:195–203.
- Turk, J. L., and Waters, M. F. R., 1969, Cell-mediated immunity in patients with leprosy, *Lancet* 2:243–246.
- Turk, J. L. and Waters, M. F. R., 1971, Immunological significance of changes in lymph nodes across the leprosy spectrum, *Clin. Exp. Immunol.* 8:363–376.
- Turk, J. L., Badenoch-Jones, P. and Parker, D., 1978, Ultrastructural observations on epithelioid cell granulomas induced by zirconium in the guinea pig, *J. Pathol.* 124:45–49.
- Wager, O., 1969, Immunological aspects of leprosy with special reference to autoimmune diseases, *Bull. WHO* 41:793–804.
- Waldorf, D. S., Sheagren, J. N., Trautman, J. R., and Block, J. B., 1966, Impaired delayed hypersensitivity in patients with lepromatous leprosy, *Lancet* 2:773–776.
- Wemambu, S. N. C., Turk, J. L., Waters, M. F. R. and Rees, R. J. W., 1969, Erythema nodosum leprosum: A clinical manifestation of the Arthus phenomenon, *Lancet* 2:933–935.

Host Resistance to Malaria

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1. INTRODUCTION

Various aspects of resistance to malaria have been reviewed in recent years (K. N. Brown, 1976a,b; Miller and Carter, 1976; Luzzatto, 1979; Pasvol and Weatherall, 1980). This chapter will therefore deal only with those papers appearing since or with arguments relating to earlier work not hitherto considered. It will be concerned with resistance of the vertebrate host only.

Central to our understanding of host resistance is recognition of the many levels of biological, structural, and antigenic complexity shown by malaria parasites and the host cells they infect, as well as the quantity and duration of the antigenic stimulation they provide. Not surprisingly for an obligate parasite dependent on an insect vector, resistance is rarely absolute, involves many factors, and is dynamic, providing a fluctuating balance for survival of both host and parasite populations geared appropriately to the dynamics of the vector. Most of this review will be concerned with the parasite rather than with the host response as such, for it is in this area that most of the important research advances have been obtained, thus making it possible to start to delineate details of both innate and acquired host resistance. Innate resistance has been fully reviewed recently (Miller and Carter, 1976; Luzzatto, 1979; Pasvol and Weatherall, 1980) and will not be considered in detail here. In the next few years a new understanding of host resistance at the cellular and molecular level will emerge, and this understanding should lead to ways of manipulating resistance to man's advantage.

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2. DEVELOPMENTAL AND STRUCTURAL ASPECTS OF THE PARASITE RELEVANT TO HOST RESISTANCE

The three specializations of parasite development relevant to host resistance, the exoerythrocytic stage, the asexual erythrocytic stage, and the sexual erythrocytic stage, have a common biphasic theme, extracellular and intracellular. The extracellular phases, the sporozoite, the exoerythrocytic and erythrocytic merozoites, and even the gametes, are directly available to immune attack, although with the gametes, this attack is apparently by vertebrate host products acting within the mosquito gut. The situation with the intracellular phases is more uncertain, but information available on the intracellular asexual erythrocytic parasite demonstrates quite clearly that immunologically the host-parasite complex must be considered not only in terms of its parasite-derived component but as an antigenic and immunogenic entity of parasite plus host cell.

There is no avoiding the fact that experimental manipulation of malaria parasites can present formidable technical problems, and this is reflected in the fact that much of our knowledge is derived from descriptive electron microscopy. With the sporozoite and the asexual erythrocytic stages, some molecular detail is beginning to emerge. The ultrastructure of malaria parasites has been reviewed by Aikawa (1977), from which reference much of the structural description below is derived.

2.1. Extracellular Phases

Two of the extracellular phases, the sporozoites and merozoites, show many common structural and functional similarities. The gametes, macro and micro, are different, representing, in fact, precursors of the sporozoite/merozoite equivalent, the ookinete. The ookinete, which penetrates the mosquito gut wall, is not relevant to this discussion.

2.1.1. Sporozoites

Inoculated by mosquitoes, sporozoites are about 11 μm in length and 1.0 μm in diameter. They circulate in the blood for only a matter of minutes. Like merozoites, they are enclosed by a pellicle consisting of a plasmalemma and an inner double membrane complex with a row of subpellicular microtubules. The anterior end is cone-shaped and demarcated by three polar rings. Electron-dense rhoptries and micronemes, thought to be associated with cell penetration, are present at the anterior end. A fibrillar coat covers the sporozoite. To date, molecular and antigenic analysis of the sporozoite has been confined to the 15 nm-thick surface coat. In *Plasmodium berghei*, this appears to consist of a single protein molecular species of 42–44,000 daltons (Gwadz *et al.*, 1979b; Yoshida

et al., 1980; Potocnjak *et al.*, 1980). In *P. knowlesi*, preliminary experiments indicated a surface coat protein of 100,000 daltons, a surprising species difference which requires confirmation. *In vivo* exposed sites on these molecules have undoubted species specificity (Potocnjak *et al.*, 1980), but other structural details are unknown.

Evidence from immunization studies suggests that antigenic sites exposed on the intact sporozoite are common to many isolates of the same species (Clyde *et al.*, 1973a), but clear strain differences have been detected (Nardin *et al.*, 1979). The circumsporozoite precipitin reaction that occurs forms a taillike precipitate, a reaction that may represent an escape "capping" mechanism by the parasite. Studies with monoclonal antibodies to the 44,000-dalton *P. berghei* surface antigen showed that monovalent (Fab) antibody failed to produce this reaction but retained strong sporozoite-neutralizing activity, possibly greater than bivalent antibody. This result lends some weight to the possible role of the circumsporozoite reaction as an escape mechanism (Potocnjak *et al.*, 1980). Another characteristic of the sporozoite surface antigen is its apparently poor immunogenicity in some parasite-host combinations (Clyde *et al.*, 1973b; Gwadz *et al.*, 1979b). Repeated intravenous immunization is required to produce an appreciable antibody response, and the ability to produce this response is idiosyncratic and of short duration, sometimes in spite of repeated challenge (Gwadz *et al.*, 1979b). Reasons for this low immunogenicity require further exploration in hosts more susceptible to infection than mice to *P. berghei* sporozoites (Vanderberg *et al.*, 1968).

Although only a single antigenic component has been positively identified exposed on the surface of sporozoites, other evidence indicating heterogeneity in surface structures comes from the marked inter- and intraspecific differences in host susceptibility to sporozoites of a given *P. berghei* isolate (Vanderberg *et al.*, 1968). The proportion of sporozoites initiating liver infection varies greatly from one host species to another. If, as seems unlikely from lactoperoxidase [^{125} I] surface labeling and precipitation with immune serum, there is substantial heterogeneity in the 44,000-dalton protein within a single *P. berghei* genotype (Gwadz *et al.*, 1979b), an alternative explanation for differences in infectivity is necessary. Perhaps relevant to this question are the observations by Schulman *et al.* (1980) that serum proteins become bound to the surface of sporozoites of *P. berghei* and *P. knowlesi*, this effect being most marked with serum from the susceptible host. In addition, Danforth *et al.* (1980) and Holmberg *et al.* (1981) have shown that serum components can facilitate cell penetration by sporozoites, albeit in an artificial *in vitro* system. No information is available on this effect at the molecular level, but Gwadz *et al.* (1979a) have reported binding of normal serum to a [^{125} I]lactoperoxidase-labeled component of approximately 19,500 daltons. Neither this component nor two others similarly labeled were precipitated by immune serum. If these molecules are in fact of sporozoite origin, then

they seem to be poorly immunogenic. The sporozoites for these studies were obtained from thoracic hemocoels as well as salivary glands. Sporozoite maturation appears to involve changes in the sporozoite surface (Vanderberg *et al.*, 1972); therefore, some of the unidentified putative surface components may in fact be expressed on the surface of immature sporozoites.

The possibility of serum components aiding the homing of sporozoites to target cells and details of the interaction of these proteins at the surface of sporozoites and host target cells, either Kupffer cells or hepatocytes, is a question of considerable biological interest. Experiments by Holmberg *et al.* (1981) indicate that the invasion-enhancing component of serum in the active sporozoite penetration of the macrophage system has terminal galactose as well as mannose or glucose residues. These workers point out that mannose receptors have been identified on Kupffer cells.

2.1.2. Merozoites

Although ultrastructurally very similar to sporozoites, merozoites are more ovoid in shape. They have an electron dense compact fibrillar surface coat about 20 nm thick removable by trypsin treatment. Because of the uncertainty surrounding the mechanism of persistence of exoerythrocytic infection (Krotoski *et al.*, 1980; K. N. Brown, 1976a), it remains uncertain whether there are two types of merozoites with specificity for either liver cells or erythrocytes or a single erythrocyte-penetrating type. Merozoites are particularly difficult to isolate and maintain in a viable form, so it is not surprising that we know nothing of the molecular characteristics of their surface. Aikawa (1977) has reported that the surface coat appears to become thickened on release of the merozoite, suggesting either a conformational change and/or binding of serum proteins.

Considerable immunologic interest has centered about the means by which erythrocytic malaria parasites enter erythrocytes. Although much of the thinking behind this was, on the whole, fairly imprecise and confused (K. N. Brown, 1976b, 1977), the stimulus provided by its assumed immunologic importance has produced a body of information of great biological interest (see Section 2.2.2) although of doubtful relevance to protective immunity. Other than indirect evidence that the merozoite surface coat antigen is a variable antigen (Butcher and Cohen, 1972; K. N. Brown, 1977; Freeman *et al.*, 1980), no new information on the supposed receptor for the erythrocyte or its antigenicity has emerged since this topic was previously discussed (K. N. Brown, 1976a,b, 1977).

A widely held view is that the erythrocyte has a receptor for the merozoite (Miller *et al.*, 1979). It is most likely, however, that the merozoite bears the receptor, since it is presumably an organelle, i.e., rhoptry, response in this cell that modifies the erythrocyte membrane and initiates formation of the parasitophorous vacuole and penetration. Thus, in this reaction it appears that the

erythrocyte provides the ligand, and the merozoite the receptor. Since merozoite attachment can occur at any orientation but penetration occurs only when the conoid abuts the erythrocyte (Dvorak *et al.*, 1975), it seems probable that binding sites for the erythrocyte ligand occur over the entire surface of the merozoite, as one part of the molecular configuration of the surface coat. Apparently ligand-receptor-generated second messenger (Greaves, 1976), or the consequence of the generation of a second messenger, is functional only in the conoid region and its associated organelles. Since the weight of evidence suggests that the surface coat is a variable antigen, the erythrocyte receptor, like the sialic acid binding site on the influenza virus hemagglutinin molecule, may be constant and nonantigenic (Wiley *et al.*, 1981). Antigenic variation in malaria, like antigenic drift in influenza virus, presumably involves all exposed adjacent antigenic sites with the erythrocyte receptor region conserved, although changes in the age distribution of erythrocytes invaded, which can occur in *P. yoelii* infections, suggests that some modification of the receptor site may occur (Freeman *et al.*, 1980). [¹²⁵I]lactoperoxidase labeling experiments (Miller *et al.*, 1980) have indicated that determinants common to *P. falciparum* and *P. knowlesi* are present on the surface of *P. knowlesi* merozoites. Since both species infect man, it is possible that these common sites could be erythrocyte receptors. The evidence that glycophorin provides the ligand for *P. falciparum* (Pasvol and Weatherall, 1980) and that *P. knowlesi* invasion is reduced in Duffy-negative human erythrocytes, although not in all Duffy-negative erythrocytes (Mason *et al.*, 1977), does not preclude this interpretation. The molecular structure of the Duffy antigen and its surface distribution in relation to surface determinants such as glycophorin are unknown. However, studies on isolated merozoites like those of Miller *et al.* (1980) should be viewed with caution, since merozoites rapidly lose viability during handling.

2.1.3. Gametes

Although gametes are released only in the gut of the mosquito vector, interest in their surface antigenicity arose from the demonstration by Carter and Chen (1976) and Gwadz (1976) of the blocking of gamete fusion, and thus parasite transmission, by serum factors taken up in the blood meal, particularly if this meal was from an animal immunized with a gamete-rich preparation.

Gametogenesis has been described in *P. yoelii* (Sinden *et al.*, 1976) and *P. falciparum* (Sinden *et al.*, 1978), but the surface antigens of gametes have not been characterized. It may be significant for the understanding of transmission-blocking effects and the possible specificity of effector antibodies that, in addition to the gamete surface and its antigens, erythrocyte membrane fragments, presumably modified during the process of gamete release, may be seen attached to the gamete surface (Sinden *et al.*, 1976).

2.2. Intracellular Phases

In an immunologic context, two aspects of the infected host cell have to be considered: (1) parasite antigens, particularly any that may become exposed on the surface of infected cells, and (2) changes wrought in the host cell, particularly in the plasma membrane, that might allow direct immune attack on the parasite within and/or render the host cell isoantigenic and immunogenic.

2.2.1. Exoerythrocytic Stage

This stage is poorly understood in terms of structure and antigenicity relevant to host resistance. Improved opportunity for electron microscopy was obtained for *P. berghei* in rats by Meis *et al.* (1981) by temporary ligation of blood vessels to the majority of the liver, thus concentrating parasites in a relatively small area. In these and earlier studies on avian malaria (Beaudoin and Strome, 1972), some evidence for disorganization of the host cell was obtained. How far this is reflected at the cell surface, and whether parasite antigens become associated with the plasmalemma of the parasitized cell, as in the erythrocytic stage, are questions that, although technically difficult, urgently require investigation. Quite basic questions such as the nature of persistent exoerythrocytic infection have yet to be answered. Some immunologic aspects of this problem have been discussed elsewhere (K. N. Brown, 1976a).

2.2.2. Asexual Erythrocytic Stage

In recent years a considerable body of information has been published concerning structural and antigenic characteristics of this stage. Much of this work indicates the need to consider the infected erythrocyte in its entirety, since the presence of the developing parasite profoundly changes the structure of the host cell itself, a fact that may have important immunologic consequences. Entry of parasites into erythrocytes with induction of the parasitophorous vacuole, initial dedifferentiation followed by subsequent growth, division and maturation of merozoites, changes in erythrocyte cytoplasm and plasmalemma, disruption of the erythrocyte, and escape of merozoites, form a cycle of events taking place every 24–72 hr according to species, a cycle that frequently involves a substantial portion of the host erythron over a period of several weeks. Consequently, large amounts of both parasite and modified host material enter the host's immune system over a protracted period. To analyze this complex, to identify factors involved in the induction of protection and the targets for immune effector mechanisms, and to explain convincingly the persistence of infection, recrudescences, and repeated reinfection, is no small task in which some progress has been made. With respect to host resistance, the parasite–erythrocyte

complex can be considered under four headings, (1) entry of the parasite, (2) the parasitophorous vacuole, (3) parasite development, and (4) the erythrocyte membrane.

2.2.2a. Entry of the Parasite. Initial contact is between the merozoite surface coat and the erythrocyte; thus, our ignorance of its molecular and antigenic structure (see Section 2.1.2) and relationship to the merozoite plasma membrane leaves little room for critical discussion. As stated previously (see Section 2.1.2), evidence from serologic and monoclonal antibody studies indicates that merozoites show antigenic variation.

The process of entry has been described at the light and electron microscope levels (Dvorak *et al.*, 1975; Bannister *et al.*, 1975; Aikawa *et al.*, 1978; McLaren *et al.*, 1979). The modification of the host erythrocyte membrane that precedes interiorization of the parasite appears to be fairly localized, since the erythrocyte assumes its normal discoid shape immediately after entry (Dvorak *et al.*, 1975). Some changes in the distribution of membrane proteins, however, are detectable by freeze-fracture soon after entry (McLaren *et al.*, 1979). It has been suggested (Kilejian, 1976) that a histidine-rich protein may be concerned with modification of the host membrane during entry, but there is no direct evidence on this point. Recent evidence from immunization studies with histidine-rich protein isolated from *P. lophurae* (McDonald *et al.*, 1981) has failed to substantiate the earlier suggestion (Kilejian, 1978) that during entry this protein may be exposed to antibody binding. Apart from the freeze-fracture studies of McLaren *et al.* (1979), we have no information on the surface membrane of erythrocytes during and immediately after entry. It would be surprising, however, if formation of the parasitophorous vacuole did not produce some secondary effect in the erythrocyte surface.

2.2.2b. Parasitophorous Vacuole. When first formed, the parasitophorous vacuole is virtually devoid of protein components (McLaren *et al.*, 1979); these begin to appear as the parasite develops (McLaren, 1979; Langreth, 1977). Since mature erythrocytes are incapable of protein synthesis, all the proteins are presumably of parasite origin, although the possibility that they are derived from the erythrocyte membrane by structures described below cannot be excluded. Expansion of the sealed parasitophorous vacuole appears to result from the action of the so-called microspheres of the parasite (Bannister *et al.*, 1975).

A feature of parasitized erythrocytes is the appearance of membrane-bound bodies ('Maurers' clefts') which have the same intramembraneous particle distribution as the parasitophorous vacuole and are probably derived therefrom (Aikawa *et al.*, 1975). These authors tentatively concluded from evidence based on high-voltage electron microscopy and stereoscopy of thick sections that the clefts extended to the erythrocyte plasmalemma and appeared to be continuous with it. Thus, this as yet unconfirmed and fairly limited electron microscope evidence indicates how integral membrane proteins could move from the para-

sitophorous vacuole to the erythrocyte surface and/or in the opposite direction. The question of parasite-derived components appearing in the erythrocyte membrane is discussed below.

2.2.2c. The Parasite. Soon after entry, the rhoptries, microspheres, and conoid and membrane-associated structures characteristic of the merozoite differentiate, and the parasite begins to grow within an expanding parasitophorous vacuole. Later nuclear and cell division and schizogony begin with differentiation into merozoites. It is evident from ultrastructural analysis that these various phases of maturation are structurally very different and therefore also likely to differ antigenically. To date, analysis of potential antigens has been confined largely to proteins. A number of studies (Deans *et al.*, 1978; Kilejian, 1980b; Freeman *et al.*, 1980; Perrin *et al.*, 1981b; Newbold *et al.*, 1982a) have shown differences in protein and antigen synthesis at different phases of the parasite maturation cycle.

The most complete study of protein synthesis through maturation is that of Newbold *et al.* (1981) who were able to establish with *P. chabaudi* that synthesis of certain polypeptides was confined to particular phases of maturation. At least three polypeptides were apparently confined to the ring stage, 12 to trophozoites, and six to the mature trophozoite–schizont stage. None of the studies has yet yielded published information on antigens of proven protective activity *in vivo*. *In vitro* inhibition of multiplication of *P. falciparum* has been demonstrated by three monoclonal antibodies reacting with polypeptides of 36,000, 41,000, and 96,000 molecular weight (Perrin *et al.*, 1981a). One of these monoclones was derived from a mouse immune to *P. berghei* rather than *P. falciparum*. The significance of *in vitro* inhibition in a *P. falciparum* system for protection *in vivo* has yet to be established. There are a number of unresolved questions concerning the structure and functioning of erythrocytes infected with *P. falciparum* under *in vitro* conditions (see Section 2.2.2d). Epidemiologic evidence (Wilson and Phillips, 1976) indicates that there is no simple direct correlation between *in vitro* inhibition and protection.

Kilejian (1980a) drew attention to a protein unusually rich in histidine synthesized by *P. falciparum*. She suggested some homology with the so-called histidine-rich protein described in *P. lophurae* and which may be involved in erythrocyte penetration (see above). The evidence for this homology at present rests on unquantified electron microscopic observation. The fact that the so-called K– isolates of *P. falciparum* (see below) can multiply *in vitro* without apparently synthesizing this protein (Kilejian 1980b) argue either against homologies between the *P. falciparum* and *P. lophurae* histidine-rich protein or against its role in erythrocyte penetration by *P. falciparum*.

2.2.2d. Surface of Infected Erythrocytes. Structural changes in the surface membrane of infected erythrocytes have been described using a variety of techniques, including electron microscopy (Aikawa, 1977; Langreth, 1977; Langreth

and Reese, 1979; McLaren *et al.*, 1979; K. N. Brown *et al.*, 1982), biochemical analysis (Weidekamm *et al.*, 1973; Wallach and Conley, 1977; Trigg *et al.*, 1977; Schmidt-Ullrich and Wallach, 1978; Schmidt-Ullrich *et al.*, 1979, 1980; Chaimanee and Yuthavong, 1979), and serologic techniques (I. N. Brown *et al.*, 1968a; K. N. Brown *et al.*, 1970b, 1980; Tosta and Wedderburn, 1980). Many of these studies have distinguished between parasitized and unparasitized cells from infected animals, whereas others have relied on erythrocytes from uninfected animals as controls. Although there is much evidence that the surfaces of parasitized cells are selectively modified, other studies by Howard *et al.* (1980a,b), using lactoperoxidase-catalyzed radioiodination, labeling with periodate-sodium borohydride, and galactose oxidase, failed to detect differences peculiar to *P. berghei*- and *P. yoelli*-parasitized cells. Shear *et al.* (1979) also found a lack of selectivity in an immune phagocytosis assay. It is quite likely that the level and duration of infection have much to do with the sensitivity of discrimination that can be achieved between infected and uninfected erythrocytes from the same animal, since disrupted parasites release cytotoxic agents (Holz *et al.*, 1977), and circulating immune complexes bind to the surface of uninfected cells (Facer, 1980).

Ultrastructural changes peculiar to infected erythrocytes have been reviewed by Aikawa (1977). These include the formation of caveolae, sometimes with associated vesicles, as with *P. vivax*, and excrescences or "knobs" as in *P. falciparum*. Changes in the distribution of intramembranous particles in the surface membrane have been demonstrated by freeze-fracture (McLaren *et al.*, 1979). Part of these changes can be attributed to the dissociation of the spectrin/actin network (Wallach and Conley, 1977; Elgasaeter and Branton, 1974). The presence of antigen with apparent parasite specificity has been detected as exposed to antibody action on intact parasitized cells by serological and ultrastructural techniques in *P. knowlesi*, *P. falciparum*, and *P. berghei* (K. N. Brown *et al.*, 1970b; Langreth and Reese, 1979; K. N. Brown *et al.*, 1982), by metabolic labeling and surface immunoprecipitation with *P. chabaudi* (Newbold *et al.*, 1982b), and by metabolic labeling and immunoprecipitation after cell fractionation with *P. knowlesi* (Schmidt-Ullrich *et al.*, 1980). Technical and interpretational problems beset all these studies, but there is no doubt that the exquisite specificity of the schizont-infected cell agglutination test and opsonization test with *P. knowlesi* (I. N. Brown *et al.*, 1968a; K. N. Brown *et al.*, 1970b) strongly favors the idea that parasite-coded proteins are present with exposed sites on the membrane.

Four parasite-derived glycoprotein antigens attributed to the erythrocyte surface by metabolic labeling and immunoprecipitation have molecules of 130, 80, 65, and 50 kilodaltons in rhesus cells infected with *P. knowlesi* (Schmidt-Ullrich *et al.*, 1980). By metabolic labelling and surface immunoprecipitation with apparently intact cells parasitized with *P. chabaudi* and *P. berghei* (New-

bold *et al.*, 1982b) and sodium boro [^3H]hydride surface labeling in *P. chabaudi* (Newbold *et al.*, 1982b) and *P. falciparum* (Kilejian 1980b), a glycoprotein of 210,000 daltons was shown to be exposed on the surface. In *P. chabaudi*, minor components of 71,000 and 58,000 daltons were also detected in some experiments. The discrepancy between the results with *P. knowlesi* and the other species is surprising and suggests that *P. knowlesi* should be investigated by surface immunoprecipitation and surface labeling techniques. The exposed determinants on the 210,000-dalton glycoprotein have species specificity (Newbold *et al.*, 1982b). Whether it shows intraspecific antigenic variation as seems possible from serologic and serum transfer studies (K. N. Brown *et al.*, 1970b; Welde and Diggs, 1978; McLean *et al.*, 1982) remains to be determined.

Host cell isoantigens can become exposed on the surface of infected erythrocytes (K. N. Brown *et al.*, 1982), and modified host macromolecules may perhaps take on a new antigenicity. Formal proof of the presence of parasite-coded antigen is required where the parasite origin of the antigen and the intact state of the parasitized cell have been established beyond question. A possible mechanism for the introduction of parasite-coded antigen into the membrane is described above.

The degree to which the various ultrastructural modifications observed in the surface of erythrocytes parasitized with different species of parasite (Aikawa, 1977) are reflected in surface antigenicity or isoantigenicity is uncertain. The caveolar-vesicle complexes observed with the *P. vivax* type of parasite may be related to the fact that those species infect predominantly reticulocytes. The spectrin/actin network of reticulocytes is incomplete, and reticulocytes can show lateral migration of bound ligands (Tokuyasu *et al.*, 1979). With *P. vivax*-infected cells, uptake of cationized ferritin into vesicles has been demonstrated (Aikawa *et al.*, 1975). With *P. berghei*-infected reticulocytes, aggregation, shedding, and pinocytosis of bound antibody occurs (K. N. Brown *et al.*, 1982). By absorption experiments, K. N. Brown *et al.* (1982) produced evidence indicating that in *P. berghei*-infected reticulocytes there was a close juxtaposition of parasite-specific antigen and erythrocyte isoantigen exposed at the cell surface, an observation possibly of significance for protective immunity (see Section 4.3.1).

A number of parasites, notably *P. falciparum* and *P. malariae* of man which parasitize predominantly mature erythrocytes, produce so-called excrescences or knobs on the erythrocyte membrane. These are clearly areas where the membrane is substantially modified and binding of antibodies from immune serum is localized (Langreth and Reese, 1979).

An unresolved aspect of the parasite-erythrocyte complex is the role of the histidine-rich protein (Kilejian, 1980a,b). In *P. falciparum*, this protein is synthesized by trophozoites and appears to be concerned with the knob formation. Biosynthesis by trophozoites does not occur in knobless (K-) sublines of *P. falciparum* (Kilejian, 1980b). The K- parasites are rarely seen *in vivo*, but in

vitro they multiply at least as well as K+ cells (Langreth and Reese, 1979); K- cells apparently fail to combine with antibodies *in vitro*. The specificity of these antibodies which could be either antiparasite or anti-modified-host components is not clear, but they may include antibodies to the histidine-rich protein itself (Kilejian, 1980a). Knobs are absent from erythrocytes infected with *P. falciparum* gametocytes, thus implying that synthesis of histidine-rich protein is repressed when maturation is directed towards gametogenesis.

The role of knobs in the parasite-erythrocyte economy is not known, but, as areas of special membrane modification, they are apparently not required for effective *in vitro* growth. This fact may imply that the surface membrane of *in vitro*-maintained erythrocytes is of itself sufficiently abnormal and modified to allow efficient parasite metabolism, and relatively free flow of macromolecules through the erythrocyte membrane may occur as the parasite matures, a consideration of importance in the interpretation of *in vitro* immunologic and biochemical studies (see above).

It is apparent from these studies that the surface of parasitized erythrocytes is neither structurally nor immunologically normal. Surface immunogenicity probably involves both parasite-derived and isoantigens. As yet uninvestigated is how far this situation may be further modified in humans suffering from the wide range of hemoglobin and metabolic abnormalities which show such a striking correlation in their distribution with that of malaria. Such pathologies can in themselves modify erythrocyte surface structure (Kahan *et al.*, 1978; Riggs and Ingram, 1977; Maniatis and Bertles, 1978), modifications that may have additive or synergistic effects with the changes induced by the parasite, conceivably leading to greater immunogenicity (see Section 4.3.1).

Certain antigens, whose location in the intact parasitized cell has not been clearly established, are apparently released either as merozoites escape or during the later stages of parasite maturation (Wilson and Bartholomew, 1975; McColm *et al.*, 1977; Langreth and Reese, 1979; K. N. Brown *et al.*, 1982). Some appear to be associated with membranous material (McColm *et al.*, 1977; Wilson and Ling, 1979) including the La₁ antigen of *P. falciparum* and the soluble and curiously poorly immunogenic S-antigens. The escape of merozoites must result in release into the immune system of an antigenic complex of erythrocyte membrane, parasitophorous vacuoles, residual pigment body and associated membranes, the membrane-bound cytoplasmic "clefts," as well as soluble products enclosed therein. Thus, the identification, origin, location, and specificity of the antigenic components in the erythrocyte complex, in addition to those actually located on or in the parasite, is a matter of some importance.

2.2.3. Gametocytes

Several structural features of gametocytes are possibly relevant to the immunology of the infection. Gametocytes are enclosed in a parasitophorous

vacuole, and their cell membrane is double layered. Osmiophilic bodies, apparently similar to rhoptries, are present in the cytoplasm, with a narrow duct leading to the inner membrane of the parasite plasmalemma (Aikawa, 1977). Electron microscopic evidence (Sinden *et al.*, 1976, 1978) indicates that these organelles are concerned with dissociation of the parasitophorous vacuole and erythrocyte membrane during gametogenesis, after ingestion by the mosquito. As mentioned previously, it may be relevant to the studies on transmission blocking (see Section 4.3.2) that the formation and release of gametes do not only involve expansion of the gametocyte membrane, but remains of the erythrocyte membrane can frequently be seen attached to the surface of the gametes. Thus, again, the parasite surface presents not only parasite-derived antigens but possibly isoantigens of attached modified erythrocyte membrane to the surrounding medium.

Erythrocytes infected with gametocytes generally develop ultrastructural modifications similar to those infected with asexual parasites. A notable exception is provided by *P. falciparum* gametocytes, where the erythrocyte membrane lacks the knobs characteristic of cells containing the asexual parasite. Analogy with the K- cells described above raises the interesting possibility that gametocytes do not synthesize the histidine-rich protein characteristic of K+ asexual forms (see Section 2.2.2).

3. CLINICAL MANIFESTATIONS OF IMMUNITY

It is almost impossible to define when man becomes immune to malaria, since all age groups under holoendemic conditions carry parasites (Molineaux and Gramiccia, 1980). Immunity appears essentially to be the ability to maintain parasitemias at subpathogenic levels. This state takes some years to develop (McGregor, 1965), with timing depending much on the epidemiologic situation. It may on occasion, such as pregnancy (Gilles *et al.*, 1969; McGregor, 1978; van Zon and Eling, 1980), cease to be fully effective. The reason why protective immunity is so slow to develop is unclear. It may be a function of the facts that a variety of antigen genotypes are present in any given area (Wilson, 1980, Wilson, personal communication) and that the antigens involved may be poorly immunogenic (see Section 2.1.1) or may include altered self components (see Sections 2.2.2; 4.3.1) generating a partly autoimmune response which tends to be rapidly suppressed. At the same time, malaria is far from being the only pathogen present in the environment; thus, separation of the malaria component from other factors carries a degree of uncertainty.

Not surprisingly, most of the obvious untoward consequences of immunity, and thus most of the investigations, relate to the erythrocyte, erythropoiesis, and the spleen. Changes in the erythrocyte picture related to malaria infection have

been the subject of several recent studies. Children exposed to holoendemic malaria are often direct Coombs' test positive (Facer *et al.*, 1979). This positivity can partly, at least, be attributed to *P. falciparum* schizont-specific antigen-IgG complexes adsorbed to the surface of nonparasitized erythrocytes (Facer, 1980), especially after antimalarial treatment (Facer *et al.*, 1979; Woodruff *et al.*, 1979) or malaria crisis. It probably contributes significantly to anemia at these times. Other effects related to the presence of circulating immune complexes appear to be glomerulonephritis (Bhamarapravati *et al.*, 1973), high immunoglobulin levels (Topley *et al.*, 1973), and the presence of rheumatoid factor (Greenwood *et al.*, 1971). Specific antierythrocyte antibodies have been detected in malarious subjects (Facer, 1980), but whether they are direct results of malaria is uncertain. Immunoglobulin binding to all erythrocytes in *P. yoelii*-infected mice has been demonstrated experimentally from very early in infection, before appreciable immune complexes would be expected to present, although this possibility cannot be excluded (Hunter *et al.*, 1980). In addition, binding of cold isohemagglutinins selectively to parasitized erythrocytes has also been shown (K. N. Brown *et al.*, 1980). These authors speculated that these antibodies might be in part responsible for the immunosuppression characteristic of high parasitemia, since cold isohemagglutinins are known to be cytotoxic for monocytes (Pruzanski and Delmage, 1977), and immunosuppression appears to derive primarily from an adherent cell population (Weinbaum *et al.*, 1978; Morges and Weidanz, 1980). Such antibodies easily elute from cells to which they bind, thus complicating *in vitro* interpretation.

Another cause of anemia in partially immune subjects is dyserythropoiesis (Abdalla *et al.*, 1980). The reason for this deficiency is unknown, but the condition rapidly resolves after antimalarial treatment. No explanation, immunologic or otherwise, is forthcoming at present for this important observation. One immunologic possibility is that the antilymphocytotoxic antibodies that have been reported in malaria (Wells *et al.*, 1980), as well as being potentially immunosuppressive, react with the differentiating erythroid cells. Both types of cell are known to have some common antigenicity (Robinson *et al.*, 1981). Alternatively, cytotoxic antibody action on T cells could disorganize erythropoiesis (Cline and Golde, 1979). Conceivably, the antilymphocyte response is generated initially by a response to reticulocytes modified by the presence of a developing parasite within or to lytic factors released from parasites (Holz *et al.*, 1977) acting on leucocytes and rendering them isoantigenic.

Other immunologic conditions that arise in a minority of individuals exposed to malaria are tropical splenomegaly and chronic progressive renal lesions. Tropical splenomegaly represents an aberrant immunologic response to recurrent malaria infection; it is normally rare, but in Papua, New Guinea, it affects 80% of some tribal groups. Studies by Crane *et al.* (1977) and Crane (1980) have shown that immunity to malaria develops normally, but the tendency for the

gross splenic enlargement of nonimmunes to be reduced as immunity develops does not occur. The IgM levels continue to rise throughout life. Much of the IgM is immunofluorescent malaria antibody, although rheumatoid factor and cold agglutinins also contribute. The disease apparently has a genetic basis, and whether it relates in some way to responses to antigens involved in protection is unknown. The same question arises with chronic progressive renal lesions sometimes found associated with *P. malariae* infection. Unlike tropical splenomegaly, this condition does not resolve with antimalarial treatment (World Health Organization, 1972).

4. PROTECTIVE IMMUNITY

Since a previous review of acquired resistance to malaria (K. N. Brown, 1976a), some progress has been made in relating acquired resistance to specific antigens and antigenic sites of parasites or parasitized cells. Nevertheless, in general, our knowledge of parasite antigenicity is still very fragmentary (see Section 2), particularly with regard to the structure and interrelationship of exposed surface antigens.

4.1. Sporozoite Stage

Most of our knowledge concerning ant sporozoite immunity comes from studies on one model, *P. berghei* in the mouse, cited in Section 2.1.1. Careful investigation over a period of years, notably by Nussenzweig and her associates, has built up a considerable body of knowledge.

Immunization with X-irradiated sporozoites incapable of cell division apparently generates two levels of protective response. The first, a direct anti-sporozoite action, is primarily antibody mediated (Nussenzweig *et al.*, 1969; Potocnjak *et al.*, 1980) and directed at the surface coat protein (see Section 2.1.1). Neutralization of sporozoites by serum from immune animals (Nussenzweig *et al.*, 1969) and by monoclonal antibodies (Yoshida *et al.*, 1980) has established quite clearly a protective role for antibody. However, these experiments failed to explain why only intravenous immunization appears effective (Spitalny and Nussenzweig, 1972; Gwadz *et al.*, 1979b) or the apparent difficulty shown by natural hosts in producing and sustaining an effective antibody response (Clyde *et al.*, 1973a,b). Epidemiologic evidence demonstrates that virtually all age groups, even in holoendemic areas, can become infected with malaria parasites. The possibility of genetic and antigenic variability in the sporozoite surface antigen was discussed in Section 2.1.1 and could be an important factor in determining the efficiency of protection. In addition, under conditions of natural transmission, the immunosuppressive effects of high erythrocyte

parasitemia might tend to inhibit the development of an antisporozyte response (Orjih and Nussenzweig, 1979).

The second level of protective response generated by irradiated sporozoites appears to involve the exoerythrocytic stage in the liver (Bafort *et al.*, 1978).

4.2. Exoerythrocytic Stage

Our knowledge of possible protective immune reactions to the exoerythrocytic stage of mammalian malaria is extremely limited (see K. N. Brown, 1976a). The experiments of Bafort *et al.* (1978) in which infected liver tissue containing exoerythrocytic forms was transferred to the peritoneal cavity of either irradiated sporozoite-vaccinated or normal recipients indicate that such immunity can exist. Graham *et al.* (1973) have demonstrated with *P. fallax* that antiexoerythrocytic immunity occurs in avian malaria. This immunity is in part antibody mediated and may involve antimerozoite antibodies. These two isolated sets of data suggest that the experimentally very difficult exoerythrocytic stage is an area much in need of investigation and against which appreciable immunity may develop.

4.3. Erythrocytic Stage

4.3.1. Asexual Parasite

It is clear from Section 2.2.2 that the antigenic stimulation provided by the asexual stage is both complex and quantitatively massive. The range of antigenic macromolecules derived from the various maturation phases of the parasite, the changes the parasite imposes on the host cell itself, genetic differences among "strains" as exemplified by the S-antigen complex (Wilson, 1980), and intra-strain antigenic variation (I. N. Brown *et al.*, 1968a; Welde and Diggs, 1978; McLean *et al.*, 1982) all add to the range of antigenic stimulation occurring under conditions of natural challenge. These considerations render immunologic data on immune responses to malaria, carried out without reference to the antigenic structure, variability, and specificity of the particular species and isolates used, extremely difficult and not infrequently impossible to interpret. Only the broadest conclusions can be drawn from immunologic manipulations, even when, as is not always the case, parasitemias are carefully monitored. Crude estimates of antibody responses such as the fluorescent antibody test applied to blood smears are so insensitive as to be of little value in the dissection of subtle and highly specific responses that undoubtedly characterize protective immunity. It should be emphasized that at present we have absolutely no *in vitro* correlate for protective immunity; the nearest we have is the knowledge that in the presence of high-titer antibodies to a particular intrastrain antigenic variant, that

particular variant does not multiply extensively in the blood (K. N. Brown *et al.*, 1970a,b; Phillips *et al.*, 1970).

Another factor in the interpretation of immunologic data in relation to malaria is the fact that most immunologic manipulations, for example, T-cell ablation or inhibition of immunoglobulin synthesis by heavy-chain isotype suppression, are usually in the last analysis only quantitative and selective within the total response against which they are directed (Galli and Droge, 1980; Lawton *et al.*, 1972; Manning, 1975). The systems in which these techniques were developed involved antigenic stimulation several orders of magnitude in amount and complexity less than that provided by malaria infection. One has only to examine the gross changes in the spleens from animals infected with malaria to appreciate the extreme caution required in the interpretation of conventional immunologic manipulations. In addition, experimenters also sometimes appear not to appreciate the fact that the characteristics of the malaria infection studied may greatly influence the result obtained. Laboratory isolates of malaria parasites which are uniformly lethal or which produce a short infection followed by a sterilizing immune response are behaving in a way that would not be viable under natural conditions of transmission. The responses they are evoking are likely to be qualitatively as well as quantitatively different from the more subtle relationships of a naturally balanced host-parasite relationship which allows for survival of both host and parasite populations.

Broad conclusions about protective immunologic responses can be deduced from the literature. They are clearly T-cell dependent (I. N. Brown *et al.*, 1968b; Stechshulte, 1969; K. N. Brown *et al.*, 1976; Roberts *et al.*, 1977; McDonald and Phillips, 1978; Jayawardena *et al.*, 1978, 1979; Grun and Weidanz, 1981) and usually and probably always B-cell dependent (Roberts *et al.*, 1977; McDonald and Phillips, 1978; Hunter *et al.*, 1979; Jayawardena *et al.*, 1978; 1979; Roberts and Weidanz, 1979; Freeman *et al.*, 1980). Macrophages probably play a significant role in protection (K. N. Brown *et al.*, 1970b; Tosta and Wedderburn, 1980).

Apparent exceptions to the B-cell dependence of protective immunity have come from work on *P. gallinaceum*-infected bursectomized chickens (Rank and Weidanz, 1976) and M-suppressed *P. chabaudi*-infected mice (Grun and Weidanz, 1981). However, in view of the contrary evidence from the other studies cited above, these results need to be reexamined using sensitive assays for the production of specific antibody against defined surface antigens of merozoites or parasitized erythrocytes. The species and strain specificity of protective immunity to malaria parasites in spite of the presence of many common antigens argues against nonspecific T-cell or T-cell/macrophage mediators being responsible for protection. Direct T-cell cytotoxicity has not been demonstrated in malaria and indeed is unlikely, at least in species parasitizing mature erythrocytes.

Another characteristic frequently attributed to malaria parasites is that of a polyclonal B-cell activator (Weinbaum *et al.*, 1978; Freeman and Parish, 1978; Rosenberg, 1978). This conclusion is dependent on the early detection by non-specific means of increases in Ig+ cells and of antibody synthesis to xenogenic erythrocytes or haptenated xenogenic, usually sheep, erythrocytes. No serious attempt has been made to establish whether the malaria parasite-erythrocyte complex has antigens that cross react with the detector systems used or with common parasite and environmental antigens to which the host is already sensitized. The appearance of antibodies directed specifically against Forssman antigen in *P. berghei* infections (Shear *et al.*, 1979) suggests the likely presence of antigens of this type in the parasite/host cell complex and gives added point to the need to examine the specificity of the systems used in detecting possible polyclonal activation.

In general, it has to be said that no new concepts to explain protective immunity to asexual erythrocytic malaria have emerged since this was previously reviewed (K. N. Brown, 1971, 1976a) and discussed by K. N. Brown *et al.* (1976). This hypothesis, that protection is primarily mediated through intrastrain variant-specific antibody responses and that clinical immunity involves T-cell recognition of an antigenic configuration common to variants, still awaits verification. Recent knowledge of the changes in the erythrocyte membrane and the apparent association of parasite and isoantigens at the erythrocyte surface (see Section 2.2.2) suggests that the configuration recognized by T cells may be a combined variable parasite antigen-isoantigen association. Such an interpretation would explain both the chronicity of malaria infection, the need usually for prolonged infection or draconian immunization methods to develop protective immunity, and the rapidity with which immunity tends to wane once an infection has been terminated. Evidence in support of this interpretation has come from experiments by K. N. Brown and Hills (1979, 1981) using phenylhydrazine treatment to mimic the massive erythrocyte modification and destruction of malaria, which significantly enhanced protective immunity, and from immunization with xenogenic erythrocytes using the Playfair-Marshall-Clarke (1973) system for generation of antierythrocyte autoimmune responses (Jarra, 1982). This working hypothesis, however, requires investigation at the cellular and molecular level in relation to the antigenic structure of parasitized erythrocytes and the specificities of antibodies combining with the surface of infected erythrocytes or merozoites.

4.3.2. Gametocytes and Gametes

The scant evidence concerning the immune response to gametocytes was reviewed previously (K. N. Brown, 1976a). Recently, interest has concentrated on the concept of transmission blocking through inhibition of gamete fusion in

the mosquito gut by serum factors taken in with the infective blood meal (Gwadz, 1976; Carter and Chen, 1976; Gwadz *et al.*, 1979a; Mendis and Targett, 1979).

Although monoclonal antibodies capable of partially inhibiting gamete fusion have been obtained (Rener *et al.*, 1980), it has still not been conclusively proved that in infected or immunized animals it is antibody and not some other serum factor, perhaps released from T-lymphocytes or activated macrophages, that inhibits gamete fusion. In the experiments reported, the test animals had an active asexual erythrocytic infection with associated immune responses. The specificity of either the monoclonal antibodies obtained or putative serum antibodies, i.e., whether they are directed against gamete antigens or modified erythrocyte membrane remnants attached to the gametes (Sinden *et al.*, 1976), has yet to be established. This question is of some importance for the assessment of this new approach to malaria control.

5. THE PRESENT POSITION CONCERNING THE ARTIFICIAL GENERATION OF RESISTANCE

The current investment on research into the immunology of malaria can be expected to answer two questions: Is vaccination against malaria possible? If not, can the knowledge gained be turned to advantage in other ways for the reduction of mortality and morbidity from malaria?

5.1. Vaccination

Vaccination will require, first and foremost, the preparation of a safe vaccine which can be stored, distributed, and administered under much less than ideal conditions, which gives substantial protection, and yet is relatively cheap. This is a pretty formidable requirement for a disease which is itself incapable of conferring protection against subsequent natural challenge without repeated reexposure.

5.1.1. Sporozoite Vaccine

On the basis of present evidence, the chances of producing a pure antigen with some protective properties seem most hopeful for the sporozoite-derived surface antigen. One would expect that production of such an antigen in pure form is within the scope of modern genetic biotechnology. Questions that need to be answered before this approach can be considered as having a likely practical outcome can be summarized as follows.

Is the surface coat antigen constant for all genotypes of a species, and if not, how many serotypes exist? This question is now susceptible to analysis.

Can immunogenicity be improved so that (1) antigen can be given intramuscularly or subcutaneously and (2) vaccination will confer complete protection on the great majority of subjects, since such immunization would confer absolutely no immunity against a breakthrough erythrocytic infection? This problem should be investigated in model systems in which sporozoites have a high infectivity rate.

5.1.2. Asexual Erythrocytic Stage Vaccine

At the moment prospects in this area look fairly bleak. No protective antigen has been described, and published immunization procedures so far have depended on the use of crude antigen sources much contaminated with host material in association with adjuvants either not acceptable or not proven acceptable in man. Questions requiring an answer are as follows.

Is there a parasite-derived antigen that in isolation confers protection against natural infection or a good laboratory equivalent?

How far if at all are erythrocyte-derived components involved in the generation of protection?

How specific is this protection? How much genotypic antigenic variability (as distinct from phenotypic antigenic variability) is present in the parasite gene pool for each species of human parasite?

These problems require an answer before design and techniques for production of a vaccine are given serious consideration.

5.1.3. Antigamete Vaccine

Clearly, the first consideration must be to define the nature and specificity of the gamete fusion-inhibitory agent. If a gamete-specific surface antigen is involved, then the problems would appear to be in principle similar to those of the sporozoite vaccine, i.e., variability, immunogenicity, and techniques for antigen synthesis.

5.2. Alternatives to Vaccination

Chemotherapy and vector control have a long and, given the truly formidable nature of the problem in many areas, an amazingly successful history. The question that can be legitimately asked of malaria immunology is whether it can contribute to increasing the effectiveness of these agents. There are two possible areas in which immunology can be expected to contribute: the development of an *in vitro* correlate for protective immunity which can be used as a field assay (such

a correlate would be invaluable for the development of rational chemotherapy and control programs.), and by increasing our knowledge of the parasite–host interface, thus aiding the development of new chemotherapeutic agents.

Both of these approaches, the possibility of vaccination and improvement in the effectiveness of nonimmunologic control techniques, require much hard-headed thinking and provide an intellectual challenge of the highest order. There are no simple answers.

REFERENCES

- Abdalla, S., Weatherall, D. J., Wickramasinghe, S. N., and Hughes, M., 1980, The anaemia of *P. falciparum* malaria, *Br. J. Haematol.* 46:171–183.
- Aikawa, M., 1977, Variations in structure and function during the life cycle of malaria parasites, *Bull. WHO* 55:139–156.
- Aikawa, M., Miller, L. H., and Rabbage, J., 1975, Caveola–vesicle complexes in the plasmalemma of erythrocytes infected by *Plasmodium vivax* and *P. cynomolgi*, *Am. J. Pathol.* 79:285–294.
- Aikawa, M., Miller, L. H., Johnson, J., and Rabbage, J., 1978, Erythrocyte entry by malarial parasites. A moving junction between erythrocyte and parasite, *J. Cell Biol.* 77:72–82.
- Bafort, J. M., Pryor, W. H., and Beaudoin, R. L., 1978, Malaria vaccine: The effect of sporozoite-induced immunity on exo-erythrocytic stages, *Ann. Soc. Belge Med. Trop.* 58:63–64.
- Bannister, L. H., Butcher, G. A., Dennis, E. D., and Mitchell, G. H., 1975, Structure and invasive behaviour of *Plasmodium knowlesi* merozoites *in vitro*, *Parasitology* 71:483–491.
- Beaudoin, R. L., and Strome, C. P. A., 1972, The feeding process in the exoerythrocytic stage of *Plasmodium lophurae* based upon observations with the electron microscope, *Proc. Helm. Soc. Wash.* 39:163–173.
- Bhamarapravati, N., Boonpucknavig, S., Boonpucknavig, V., and Yaemboonruang, V., 1973, Glomerular changes in acute *Plasmodium falciparum* infection, *Arch. Pathol.* 96:289–293.
- Brown, I. N., Brown, K. N., and Hills, L. A., 1968a, Immunity to malaria: The antibody response to antigenic variation by *Plasmodium knowlesi*, *Immunology* 14:127–138.
- Brown, I. N., Allison, A. C., and Taylor, R. B., 1968b, *Plasmodium berghei* infection in thymectomized rats, *Nature* 219:292–293.
- Brown, K. N., 1971, Protective immunity to malaria parasites: A model for the survival of cells in an immunologically hostile environment, *Nature* 230:163–167.
- Brown, K. N., 1976a, Resistance to malaria, in: *Immunology of Parasitic Infections*, (S. Cohen and E. Sadun, eds.), Blackwell Scientific, Oxford, pp. 268–295.
- Brown, K. N., 1976b, Specificity in host-parasite interaction, in: *Receptors and Recognition*, Volume 1, Series A, (P. Cuatrecasas and M. F. Greaves, eds.), Chapman and Hall, London, pp. 121–175.
- Brown, K. N., 1977, Invasion of erythrocytes and antigenic variation, *Bull. WHO* 55:187–189.
- Brown, K. N., and Hills, L. A., 1979, The possible role of isoantigens in protective immunity to malaria, *Bull. WHO* 57(Suppl. 1):135–138.
- Brown, K. N., and Hills, L. A., 1981, Erythrocyte destruction and protective immunity to malaria: Enhancement of the immune response by phenylhydrazine treatment, *Tropenmed. Parasitol.* 32:67–72.
- Brown, K. N., Brown, I. N., and Hills, L. A., 1970a, Immunity to malaria. I. Protection against *Plasmodium knowlesi* shown by monkeys sensitized with drug-suppressed infections or by dead parasites in Freund's adjuvant, *Exp. Parasitol.* 28:304–317.
- Brown, K. N., Brown, I. N., Trigg, P. I., Phillips, R. S., and Hills, L. A., 1970b, Immunity to

- malaria. II. Serological response of monkeys sensitized by drug-suppressed infections or by dead parasitized cells in Freund's complete adjuvant, *Exp. Parasitol.* 28:318–338.
- Brown, K. N., Jarra, W., and Hills, L. A., 1976, T cells and protective immunity to *Plasmodium berghei* in rats, *Infect. Immun* 14:858–871.
- Brown, K. N., Grundy, M. S., Hills, L. A., and Jarra, W., 1980, Cold isohaemagglutinins in *Plasmodium berghei*-infected rats reacting with parasitized reticulocytes, *Bull. WHO* 58:449–457.
- Brown, K. N., McLaren, D. J., Hills, L. A., and Jarra, W., 1982, The binding of antibodies from *Plasmodium berghei*-infected rats to isoantigenic and parasite-specific antigenic sites on the surfaces of infected reticulocytes, *Parasite Immunol.* 4:21–31.
- Butcher, G. A., and Cohen, S., 1972, Antigenic variation and protective immunity in *Plasmodium knowlesi* malaria, *Immunology* 23:503–521.
- Carter, R., and Chen, D. H., 1976, Malaria transmission blocked by immunization with gametes of the malaria parasite, *Nature* 263:57–60.
- Chaimanee, P., and Yuthavong, Y., 1979, Phosphorylation of membrane proteins from *Plasmodium berghei*-infected red cells. *Biochem. Biophys. Res. Commun.* 87:953–959.
- Cline, M. J., and Golde, D. W., 1979, Cellular interactions in hematopoiesis, *Nature* 277:177–181.
- Clyde, D. F., McCarthy, V. C., Miller, R. G., and Hornick, R. B., 1973a, Specificity of protection of man immunized against sporozoite-induced falciparum malaria, *Am. J. Med. Sci.* 266:398–403.
- Clyde, D. F., Most, H., McCarthy, V. C., and Vanderberg, P. J., 1973b, Immunization of man against sporozoite-induced falciparum malaria, *Am. J. Med. Sci.* 266:169–177.
- Crane, G. G., 1980, Tropical splenomegaly syndrome in Papua, New Guinea, in: *Tropical Immunology, Research for Development*, Seminar Three, (L. Davidson, ed.), Commonwealth Institute of Health, Sydney, p. 153.
- Crane, G. G., Gardner, A., Hudson, P., Hudson, B., and Voller, A., 1977, Malarial antibodies in tropical splenomegaly syndrome in Papua, New Guinea, *Trans. R. Soc. Trop. Med. Hyg.* 71:308–314.
- Danforth, H. D., Aikawa, M., Cochrane, A. H., and Nussenzweig, R. S., 1980, Sporozoites of mammalian malaria: Attachment to, interiorization and fate within macrophages, *J. Protozool.* 27:193–202.
- Deans, J. A., Dennis, E. D., and Cohen, S., 1978, Antigenic analysis of sequential erythrocyte stages of *Plasmodium knowlesi*, *Parasitology* 77:333–344.
- Dvorak, J. A., Miller, L. H., Whitehouse, W. C., and Shiroishi, T., 1975, Invasion of erythrocytes by malaria merozoites, *Science* 187:748–750.
- Elgasaeter, A., and Branton, D., 1974, Intramembrane particle aggregation in erythrocyte ghosts. I. The effects of protein removal, *J. Cell Biol.* 63:1018–1030.
- Facer, C. A., 1980, Direct Coombs antiglobulin reactions in Gambian children with *Plasmodium falciparum* malaria. II. Specificity of erythrocyte-bound IgG, *Clin. Exp. Immunol.* 39:279–288.
- Facer, C. A., Bray, R. S., and Brown, J., 1979, Direct Coombs antiglobulin reactions in Gambian children with *Plasmodium falciparum* malaria, *Clin. Exp. Immunol.* 35:119–127.
- Freeman, R. R., and Parish, C. R., 1978, Polyclonal B-cell activation during rodent malarial infections, *Clin. Exp. Immunol.* 32:41–45.
- Freeman, R. R., Holder, A. A., Avril, J. T., and Cross, G. A. M., 1980, Monoclonal antibodies against the rodent malarial parasite, *Plasmodium yoelii*, in: *The Host Invader Interplay* (H. Van den Bossche, ed.), Elsevier/North Holland, Amsterdam, pp. 121–124.
- Galli, P., and Droge, W., 1980, Development of cytotoxic T lymphocyte precursors in the absence of the thymus, *Eur. J. Immunol.* 10:87–92.
- Gilles, H. M., Lamson, J. B., Sibellas, M., Voller, A., and Allan, N., 1969, Malaria, anaemia and pregnancy, *Ann. Trop. Med. Parasitol.* 63:245–263.

- Graham, H. A., Palczuk, N. C., and Stauber, L. A., 1973, Immunity to exoerythrocytic forms of malaria. II. Passive transfer of immunity to exo-erythrocytic forms, *Exp. Parasitol.* 34:372–381.
- Greaves, M. F., 1976, Cell surface receptors. A biological perspective, in: *Receptors and Recognition*, Volume 1, Series A (P. Cuatrecasas and M. F. Greaves, eds.), Chapman and Hall, London, pp. 1–32.
- Greenwood, B. M., Muller, A. S., and Valkenburg, H. A., 1971, Rheumatoid factor in Nigerian sera, *Clin. Exp. Immunol.* 9:161–173.
- Grun, L. J., and Weidanz, W. P., 1981, Immunity to *Plasmodium chabaudi adami* in the B-cell-deficient mouse, *Nature* 290:143–145.
- Gwadz, R. W., 1976, Malaria: Successful immunization against the sexual stages of *Plasmodium gallinaceum*, *Science* 193:1150–1151.
- Gwadz, R. W., Carter, R., and Green, I., 1979a, Gamete vaccines and transmission-blocking in malaria, *Bull. WHO* 57(Suppl. 1):175–180.
- Gwadz, R. W., Cochrane, A. H., Nussenzweig, V., and Nussenzweig, R. S., 1979b, Preliminary studies on vaccination of rhesus monkeys with irradiated sporozoites of *Plasmodium knowlesi* and characterization of surface antigens of these parasites, *Bull. WHO* 57:(Suppl. 1):165–173.
- Holmberg, S., Schulman, S., and Vanderberg, J. P., 1981, *Plasmodium berghei*: Role of a serum factor in enhancement of *in vitro* interactions between sporozoites and hamster peritoneal macrophages, *J. Parasitol.*, 67:893–897.
- Holz, G. G., Jr., Beach, D. H., and Sherman, I. W., 1977, Octadecenoic fatty acids and their association with hemolysis in malaria, *J. Protozool.* 24:566–574.
- Howard, R. J., Smith, P. M., and Mitchell, G. F., 1980a, Characterization of surface proteins and glycoproteins on red blood cells from mice infected with haemosporidia: *Plasmodium berghei* infections of BALB/c mice, *Parasitology* 81:273–298.
- Howard, R. J., Smith, P. M., and Mitchell, G. F., 1980b, Characterisation of surface proteins and glycoproteins on red cells from mice infected with haemosporidia: *Plasmodium yoelii* infections of BALB/c mice, *Parasitology* 81:299–314.
- Hunter, K. W., Jr., Finkelman, F. D., Strickland, G. T., Sayles, P. C., and Scher, I., 1979, Defective resistance to *Plasmodium yoelii* in CBA/N mice, *J. Immunol.*, 123:133–137.
- Hunter, K. W., Finkelman, F. D., Strickland, G. T., Sayles, P. C., and Scher, I., 1980, Murine malaria: Analysis of erythrocyte surface-bound immunoglobulin by flow microfluorimetry, *J. Immunol.* 125:169–174.
- Jarra, W., 1982, Protective immunity to malaria and anti-erythrocyte autoimmunity, *Ciba Found. Symp.*, in press.
- Jayawardena, A. N., Targett, G. A. T., Leuchars, E., and Davies, A. J. S., 1978, The immunological response of CBA mice to *P. yoelii*. II. The passive transfer of immunity with serum and cells, *Immunology* 34:157–165.
- Jayawardena, A. N., Janeway, C. A., Jr., and Kemp, J. D., 1979, Experimental malaria in the CBA/N mouse, *J. Immunol.* 123:2532–2539.
- Kahane, I., Polliack, A., Rachmilewitz, E. A., Bayer, E. A., and Skutelsky, E., 1978, Distribution of sialic acids on the red blood cell membrane in beta thalassaemia, *Nature* 271:674–675.
- Kilejian, A., 1976, Does a histidine-rich protein from *Plasmodium lophurae* have a function in merozoite penetration? *J. Protozool.* 23:272–277.
- Kilejian, A., 1978, Histidine-rich protein as a model malaria vaccine, *Science* 201:922–924.
- Kilejian, A., 1980a, Homology between a histidine-rich protein from *Plasmodium lophurae* and a protein associated with the knob-like protrusions on membranes of erythrocytes infected with *Plasmodium falciparum*, *J. Exp. Med.* 151:1534–1538.
- Kilejian, A., 1980b, Stage-specific proteins and glycoproteins of *Plasmodium falciparum*: Identifica-

- tion of antigens unique to schizonts and merozoites, *Proc. Natl. Acad. Sci. U.S.A.* 77:3695–3699.
- Krotoski, W. A., Krotoski, D. M., Garnham, P. C. C., Bray, R. S., Killick-Kendrick, R., Draper, C. C., Targett, G. A. T., and Guy, M. W., 1980, Relapses in primate malaria: Discovery of two populations of exoerythrocytic stages. Preliminary note, *Br. Med. J.* 280:153–154.
- Langreth, S. G., 1977, Electron microscope cytochemistry of host–parasite membrane interactions in malaria, *Bull. WHO* 55:171–178.
- Langreth, S. G., and Reese, R. T., 1979, Antigenicity of the infected erythrocyte and merozoite surfaces in *falciparum* malaria, *J. Exp. Med.* 150:1241–1254.
- Lawton, A. R., Asofsky, R., Hylton, M. B., and Cooper, M. D., 1972, Suppression of immunoglobulin class synthesis in mice. I. Effects of treatment with antibody to M-chain, *J. Exp. Med.* 135:277–297.
- Luzzatto, L., 1979, Genetics of red cells and susceptibility to malaria, *J. Am. Soc. Hematol.* 54:961–976.
- Maniatis, A., and Bertles, J. F., 1978, Erythrocyte I-i antigens: Distribution in normal and sickle erythrocytes and relationship of i antigenicity to haemoglobin F cell content, in: *Erythrocyte Membranes: Recent Clinical and Experimental Advances*, (W. C. Kruckeberg, J. W. Eaton, and G. J. Brewer, eds.), Alan R. Liss, New York, pp. 159–168.
- Manning, D. D., 1975, Heavy chain isotype suppression: A review of the immunosuppressive effects of heterologous anti-Ig heavy chain antisera, *J. Reticuloendothel.* 18:63–85.
- Mason, S. J., Miller, L. H., Shiroishi, T., Dvorak, J. A., and McGinniss, M. H., 1977, The Duffy blood group determinants: Their role in the susceptibility of human and animal erythrocytes to *Plasmodium knowlesi* malaria, *Br. J. Haematol.* 36:327–335.
- McColm, A. A., Shakespeare, P. G., and Trigg, P. I., 1977, Release of protein by erythrocytic stages of *Plasmodium knowlesi* during cultivation *in vitro*, *Bull. WHO* 55:277–283.
- McDonald, V., and Phillips, R. S., 1978, *Plasmodium chabaudi* in mice. Adoptive transfer of immunity with enriched populations of spleen T and B lymphocytes, *Immunology* 34:821–830.
- McDonald, V., Hannon, M., Tanigoshi, L., and Sherman, I. W., 1981, *Plasmodium lophurae*: Immunization of Peking ducklings with different antigen preparations, *Exp. Parasitol.* 51:195–203.
- McGregor, I. A., 1965, Consideration of some aspects of human malaria, *Trans. R. Soc. Trop. Med. Hyg.* 59:145–152.
- McGregor, I. A., 1978, Topical aspects of the epidemiology of malaria, *Israel J. Med. Sci.* 14:523–536.
- McLaren, D. J., Bannister, L. H., Trigg, P. I., and Butcher, G. A., 1979, Freeze fracture studies on the interaction between the malaria parasite and the host erythrocyte in *Plasmodium knowlesi* infection, *Parasitology* 79:125–139.
- McLean, S. A., Pearson, C. D., and Phillips, R. S., 1982, *Plasmodium chabaudi*: Evidence of antigenic variation by the parasite during recrudescence parasitaemias in mice, *Exp. Parasitol.*, in press.
- Meis, J. F. G. M., Verhave, J. P., Jap, P. H. K., Hess, F., and Meuwissen, J. H. E. T., 1981, An ultrastructural study of developing stages of exo-erythrocytic *Plasmodium berghei* in rat hepatocytes, *Parasitology* 82:195–204.
- Mendis, K. N., and Targett, G. A. T., 1979, Immunisation against gametes and asexual erythrocytic stages of a rodent malaria parasite, *Nature* 277:389–391.
- Miller, L. H., and Carter, R., 1976, A review. Innate resistance in malaria, *Exp. Parasitol.* 40:132–146.
- Miller, L. H., Aikawa, M., Johnson, J. G., and Shiroishi, T., 1979, Interaction between cytochalasin B-treated parasites and erythrocytes. Attachment and junction formation, *J. Exp. Med.* 149:172–184.

- Miller, L. H., Johnson, J. G., Schmidt-Ullrich, R., Haynes, H. D., Wallach, D. F. H., and Carter, R., 1980, Determinants on surface proteins of *Plasmodium knowlesi* common to *Plasmodium falciparum* schizonts, *J. Exp. Med.* 151:790–798.
- Molineaux, L., and Gramiccia, G., 1980, *The Garki Project. Research on the Epidemiology and Control of Malaria in the Sudan Savanna of West Africa*, World Health Organization, Geneva.
- Morges, W., and Weidanz, W. P., 1980, *Plasmodium yoelii: The thymus-dependent lymphocytes in mice immunodepressed by malaria*, *Exp. Parasitol.* 50:188–194.
- Nardin, E., Gwadz, R. W., and Nussenzweig, R. S., 1979, Characterization of sporozoite surface antigens by indirect immunofluorescence: Detection of stage- and species-specific antimalarial antibodies, *Bull. WHO* 57(Suppl. 1):211–217.
- Newbold, C. I., Boyle, D. B., Smith, C. C. and Brown, K. N., 1982a, Stage specific protein and nucleic acid synthesis during the asexual cycle of the rodent malaria *Plasmodium chabaudi*, *Mol. Biochem. Parasitol.* 5:33–44.
- Newbold, C. I., Boyle, D. B., Smith, C. C. and Brown, K. N., 1982b, Identification of a schizont- and species-specific surface glycoprotein on erythrocytes infected with rodent malaras, *Mol. Biochem. Parasitol.* 5:45–54.
- Nussenzweig, R., Vanderberg, J., and Most, H., 1969, Protective immunity produced by the injection of X-irradiated sporozoites of *Plasmodium berghei*. IV. Dose response, specificity and humoral immunity, *Milit. Med.* 134:1176–1182.
- Orjih, A. U., and Nussenzweig, R. S., 1979, *Plasmodium berghei*: A suppression of antibody response to sporozoite stage by acute blood stage infection, *Clin. Exp. Immunol.* 38:1–8.
- Pasvol, G., and Weatherall, D. J., 1980, Annotation. The red cell and the malaria parasite, *Br. J. Haematol.* 46:165–170.
- Perrin, L. H., Ramirez, E., Lambert, P. H., and Miescher, P. A., 1981a, Inhibition of *P. falciparum* growth in human erythrocytes by monoclonal antibodies, *Nature* 289:301–303.
- Perrin, L. H., Dayal, R., and Rieder, H., 1981b, Characterization of antigens from erythrocytic stages of *Plasmodium falciparum* reacting with human sera, *Trans. R. Soc. Trop. Med. Hyg.* 75:163–165.
- Phillips, R. S., Wolstencroft, R. A., Brown, I. N., Brown, K. N., and Dumonde, D. C., 1970, Immunity to malaria, III. Possible occurrence of a cell-mediated immunity to *Plasmodium knowlesi* in chronically infected and Freund's adjuvant-sensitized monkeys, *Exp. Parasitol.* 28:339–355.
- Playfair, J. H. L., and Marshall-Clarke, S., 1973, Induction of red cell autoantibodies in normal mice, *Nature [New Biol.]* 243:213–214.
- Potocnjak, P., Yoshida, N., Nussenzweig, R. S., and Nussenzweig, V., 1980, Monovalent fragments (Fab) of monoclonal antibodies to a sporozoite surface antigen (Pb 44) protect mice against malaria infection, *J. Exp. Med.* 151:1504–1513.
- Pruzanski, W., and Delmage, K. J., 1977, Cytotoxic and cytotoxic activity of homogeneous cold agglutinins on peripheral blood monocytes, *Clin. Immunol. Immunopathol.* 7:130–138.
- Rank, R. G., and Weidanz, W. P., 1976, Nonsterilizing immunity in avian malaria: An antibody-independent phenomenon, *Proc. Soc. Exp. Biol. Med.* 151:257–259.
- Reiner, J., Carter, R., Rosenberg, Y., and Miller, L. H., 1980, Anti-gamete monoclonal antibodies synergistically block transmission of malaria by preventing fertilization in the mosquito, *Proc. Natl. Acad. Sci. U.S.A.* 77:6797–6799.
- Riggs, M. G., and Ingram, V. M., 1977, Differences in erythrocyte membrane proteins and glycoproteins in sickle cell disease, *Biochem. Biophys. Res. Commun.* 74:191–198.
- Roberts, D. W., and Weidanz, W. P., 1979, T cell immunity to malaria in the B-cell deficient mouse, *Am. J. Trop. Med. Hyg.* 28:1–3.
- Roberts, D. W., Rank, R. G., Weidanz, W. P., and Finerty, J. F., 1977, Prevention of recrudescence

- malaria in nude mice by thymic grafting or by treatment with hyperimmune serum, *Infect. Immun.* 16:821–826.
- Robinson, J., Sieff, C., Delia, D., Edwards, P. A. W., and Greaves, M., 1981, Expression of cell-surface HLA-DR, HLA-ABC and glycophorin during erythroid differentiation, *Nature*, 289:68–71.
- Rosenberg, Y. J., 1978, Autoimmune and polyclonal B cell responses during murine malaria, *Nature* 274:170–172.
- Schmidt-Ullrich, R., and Wallach, D. F. H., 1978, *Plasmodium knowlesi*-induced antigens in membranes of parasitized rhesus monkey erythrocytes, *Proc. Natl. Acad. Sci. U.S.A.* 75:4949–4953.
- Schmidt-Ullrich, R., Wallach, D. F. H., and Lightholder, J., 1979, Two *Plasmodium knowlesi*-specific antigens on the surface of schizont-infected rhesus monkey erythrocytes induce antibody production in immune hosts, *J. Exp. Med.* 150:86–99.
- Schmidt-Ullrich, R., Wallach, D. F. J., and Lightholder, J., 1980, Metabolic labelling of *P. knowlesi*-specific glycoproteins in membranes of parasitized rhesus monkey erythrocytes, *Cell Biol. Int. Rep.* 4:555–561.
- Schulman, S., Oppenheim, J. D., and Vanderberg, J. P., 1980, *Plasmodium berghei* and *Plasmodium knowlesi*: Serum binding to sporozoites, *Exp. Parasitol.* 49:420–429.
- Shear, H. L., Nussenzweig, R. S., and Bianco, C., 1979, Immune phagocytosis in murine malaria, *J. Exp. Med.* 149:1288–1298.
- Sinden, R. E., Canning, E. U., and Spain, B., 1976, Gametogenesis and fertilization in *Plasmodium yoelii nigeriensis*: A transmission electron microscope study, *Proc. R. Soc. Lond. [Biol.]* 193:55–76.
- Sinden, R. E., Canning, E. U., Bray, R. S., and Smalley, M. E., 1978, Gametocyte and gamete development in *Plasmodium falciparum*, *Proc. R. Soc. Lond. [Biol.]* 201:375–399.
- Spitalny, G. L., and Nussenzweig, R. S., 1972, Effect of various routes of immunization and methods of parasite attenuation on the development of protection against sporozoite-induced rodent malaria, *Proc. Helm. Soc. Wash.* 39:506–514.
- Stechshulte, D. J., 1969, Effect of thymectomy on *Plasmodium berghei* infected rats, *Proc. Soc. Exp. Biol. Med.* 131:748–752.
- Tokuyasu, K. T., Schekman, R., and Singer, S. J., 1979, Domains of receptor mobility and endocytosis in the membranes of neonatal human erythrocytes and reticulocytes are deficient in spectrin, *J. Cell Biol.* 80:481–486.
- Topley, E., Knight, R., and Woodruff, A. W., 1973, The direct antiglobulin test and immunoconglutinin titres in patients with malaria, *Trans. R. Soc. Trop. Med. Hyg.* 67:51–54.
- Tosta, C. E., and Wedderburn, N., 1980, Immune phagocytosis of *Plasmodium yoelii*-infected erythrocytes by macrophages and eosinophils, *Clin. Exp. Immunol.* 42:114–120.
- Trigg, P. I., Hirst, S. I., Shakespeare, P. G., and Tappenden, L., 1977, Labelling of membrane glycoprotein in erythrocytes infected with *Plasmodium knowlesi*, *Bull. WHO* 55:205–209.
- Vanderberg, J. P., Nussenzweig, R. S., and Most, H., 1968, Further studies on the *Plasmodium berghei*-*Anopheles stephensi* rodent system of mammalian malaria, *J. Parasitol.* 54:1009–1016.
- Vanderberg, J. P., Nussenzweig, R. S., Sanabria, Y., Nawrot, R., and Most, H., 1972, Stage specificity of anti-sporozoite antibodies to rodent malaria and its relationship to protective immunity, *Proc. Helm. Soc. Wash* 39:514–525.
- van Zon, A. A. J. C., and Eling, W. M. C., 1980, Pregnancy associated recrudescence in murine malaria (*Plasmodium berghei*), *Tropenmed. Parasitol.* 31:402–408.
- Wallach, D. F. H., and Conley, M., 1977, Altered membrane proteins of monkey erythrocytes infected with simian malaria, *J. Mol. Med.* 2:119–136.

- Weidekamm, E., Wallach, D. F. H., Lin, P. S., and Hendricks, J., 1973, Erythrocyte membrane alterations due to infection with *Plasmodium berghei*, *Biochim. Biophys. Acta* 323:539–546.
- Weinbaum, F. I., Weintraub, J., Nkrumah, F. K., Evans, C. B., Tigelaar, R. E., and Rosenberg, Y. J., 1978, Immunity to *Plasmodium yoelii* in mice. II. Specific and nonspecific cellular and humoral responses during the course of infection, *J. Immunol.* 121:629–636.
- Welde, B. T., and Diggs, C. L.; 1978, *Plasmodium berghei*: Biological variation in immune serum-treated mice, *Exp. Parasitol.* 44:197–201.
- Wells, R. A., Pavanand, K., Zolyomi, S., Permpnich, B., and McDermotts, R. P., 1980, Anti-lymphocytotoxic antibodies in sera of Thai adults infected with *Plasmodium falciparum* or *Plasmodium vivax*, *Clin. Exp. Immunol.* 39:663–667.
- Wiley, D. C., Wilson, I. A., and Skehel, J. J., 1981, Structural identification of the antibody binding sites of Hong Kong influenza haemagglutinin and their involvement in antigenic variation, *Nature* 289:373–378.
- Wilson, R. J. M., 1980, Serotyping *Plasmodium falciparum* malaria with S-antigens, *Nature* 284:451–452.
- Wilson, R. J. M., and Bartholomew, R. K., 1975, The release of antigens by *Plasmodium falciparum*, *Parasitology*, 71:183–192.
- Wilson, R. J. M., and Ling, I., 1979, Fractionation and characterization of *Plasmodium falciparum* antigens, *Bull. WHO* 57(Suppl. 1):123–133.
- Wilson, R. J. M., and Phillips, R. S., 1976, Method to test inhibitory antibodies in human sera to wild populations of *Plasmodium falciparum*, *Nature* 263:132–134.
- Woodruff, A. W., Ansdale, V. E., and Pettitt, L. E., 1979, Cause of anaemia in malaria, *Lancet* 1:1055–1057.
- World Health Organization, 1972, Immunopathology of nephritis in Africa, *Bull. WHO* 46:387–396.
- Yoshida, N., Nussenzweig, R. S., Potocnjak, P., Nussenzweig, V., and Aikawa, M., 1980, Hybridoma produces protective antibodies directed against the sporozoite stage of malaria parasite, *Science* 207:71–73.

Idiopathic Small Intestinal Disease in the Tropics

S. J. BAKER

1. HISTORICAL INTRODUCTION

For over two and one-half thousand years, small intestinal disease has been recognized as a health problem on the Indian subcontinent. Caraka, somewhere between the 13th and 6th centuries B.C., described “grahani vyadhi” or “small intestinal disease” in the following terms:

The patient even if not emaciated feels weakness and langour. . . . Food instead of contributing to growth issues out of the body in an upward or downward course . . . whatever food a person afflicted with grahani disease takes is improperly digested. The person repeatedly evacuates stools that are sometimes watery, sometimes dry, sometimes consisting of undigested matter and frothy, the downward wind making a loud noise at the time (Caraka Samhita, 1949).

The first description of tropical small intestinal disease in European medical literature appears to be that of “*apthoides chronica*” in Barbados (Hillary, 1759). A somewhat similar condition was observed among the Spanish in Puerto Rico between 1771 and 1776 (Abbad y Lasiera, 1959). During the 19th and first half of the 20th century, numerous reports appeared of an illness characterized by chronic nonbloody diarrhea and anemia from the Indian subcontinent, China, Indochina, Java, Cuba, and Puerto Rico. Various names were given to this illness by different investigators, including “hepatic flux” (Ballingall, 1818), “chronic diarrhea of India and the tropics” (Fayrer, 1881), “diarrhea alba” (MacLean, 1886), “psilosis” (Thin, 1897), and “hill diarrhea” (Grant, 1854).

Manson (1880) appears to have been first to apply the term “sprue” to this syndrome. This is apparently an Anglicized form of the Dutch word “sprouw,” which was applied by Ketelaer (1669) to a disease in Belgium characterized by aphthous ulceration and chronic diarrhea. The word “tropical” was added subsequently to distinguish it from the “nontropical” variety, which is now known as celiac disease or gluten-induced enteropathy.

In the first half of the 20th century, the work of Bahr (1915), Fairley (1930), Suarez (1938), and others established that tropical sprue was primarily a gastrointestinal disorder associated with steatorrhea, megaloblastic anemia, and multiple deficiency states. Bahr also demonstrated the presence of histological abnormalities in the small intestinal mucosa (Bahr, 1915), but the existence of these abnormalities continued to be a matter of controversy for many years.

With the advent of modern investigational techniques, there has been a renewed interest in, and increased understanding of, small intestinal diseases occurring in the tropics. Most of the remainder of this review will be confined to this period. For further historical details, the reader is referred to reviews by O'Brien (1971) and Cook (1978a).

2. THE SPECTRUM OF SMALL INTESTINAL DISEASE IN THE TROPICS

Residents of the tropics may be afflicted by almost any of the diseases of the small intestine that occur in the temperate areas of the world, although the relative prevalences of different diseases may vary widely. For example, in a detailed study of over 1500 patients with small bowel disease in South India over a 20-year period, this reviewer saw only four cases of Crohn's disease of the small bowel, whereas working in a similar sized institution in Canada, as many new cases may be seen in a month.

It is not the purpose of this review to deal with these conditions, which are well covered in the standard temperate-zone-oriented text books of medicine. However, in addition to these well-known temperate diseases, people living in, or visiting, the tropics are liable to develop a variety of diseases of the small intestine that are either relatively less common, or perhaps never seen, in residents of temperate climes. These latter include diseases whose etiology is well recognized such as acute bacterial infections, intestinal tuberculosis, and parasitic disorders such as hookworm, giardiasis, strongyloidiasis, and capillariasis. But over and above these clearly identifiable disorders, there is a wide spectrum of ill-defined disease of unknown origin, ranging from mild asymptomatic abnormalities of structure and function of the small bowel to a full-blown picture of intestinal malabsorption presenting with chronic diarrhea, weight loss, mega-

loblastic anemia, steatorrhea, hypoproteinemia, vitamin deficiencies, and multiple absorptive defects.

Unfortunately, there is a great deal of confusion in the literature regarding the nomenclature of conditions within this spectrum of disease. Such confusion is probably inevitable for as long as ignorance of etiology and pathogenesis persists. For the purpose of this review, the spectrum of tropical idiopathic small intestinal disease will be arbitrarily divided into a consideration of the mild asymptomatic abnormalities of structure and function, hereafter termed "tropical enteropathy" (Baker and Mathan, 1972), and symptomatic disease associated with malabsorption of two or more substances, hereafter referred to as "tropical sprue" (Klipstein and Baker, 1970). The borderland between these two is even more obscure and can probably only be profitably explored when we understand the two entities more fully.

3. TROPICAL ENTEROPATHY

3.1. Occurrence

With the application of jejunal biopsy studies, it soon became evident that morphological abnormalities of the jejunal mucosa were widely prevalent in asymptomatic people living in a number of tropical or developing countries including India (Baker *et al.*, 1962; Jeejeebhoy *et al.*, 1966), Pakistan (Russell *et al.*, 1966), Bangladesh (Lindenbaum *et al.*, 1966a), Thailand (Sprinz *et al.*, 1962; Troncale *et al.*, 1967), Vietnam (Sheehy *et al.*, 1965, 1968; Colwell *et al.*, 1968), Singapore (England and O'Brien, 1966), Australia (in Aborigines) (Walker-Smith and Reye, 1971), Iran (Nasr *et al.*, 1976), Liberia (Rhodes *et al.*, 1971), Egypt (Halsted *et al.*, 1969), Uganda (Banwell *et al.*, 1964; Cook *et al.*, 1969), Rhodesia (Zimbabwe) (Thomas *et al.*, 1976), Zambia (Cook *et al.*, 1973), Nigeria (Falaiye, 1969, 1970), Haiti (Klipstein *et al.*, 1966a; Brunser *et al.*, 1970), the Dominican Republic (Klipstein *et al.*, 1973a), Puerto Rico (Angel *et al.*, 1963; Robins *et al.*, 1967; Swanson *et al.*, 1966; Klipstein *et al.*, 1972), Mexico (Garcia, 1968), Venezuela (Roche and Layrisse, 1966), Guatemala (Schneider and Viteri, 1972), and Peru (Perea *et al.*, 1978).

3.2. Pathology

With increasing degrees of damage, the intestinal villi assume a "leaf," "ridge," or "convoluted" pattern, and finally, in the most damaged mucosa (e.g., in celiac disease), the surface is "flat" and devoid of villi (Booth *et al.*, 1962). In the tropics, fingerlike villi are rare, and the predominant form, in

apparently healthy individuals, is "leaf" or "ridge" shaped (Baker *et al.*, 1962), reflecting widespread mild damage of the intestinal mucosa. On histological section, ridges cut along their long axes give the appearance of "broad flat" villi. In addition, there is an increase in the depth of the crypts with a corresponding reduction in the height of the villus and an increase in the cellular infiltrate in the lamina propria and in the number of lymphocytes between the epithelial cells (Baker *et al.*, 1962; Swanson and Thomassen, 1965; Cook *et al.*, 1969; Halstead *et al.*, 1969; Haghighi and Nasr, 1975). Electron microscopic studies confirm the above light microscopy findings and, in addition, show an increase in lysosomes in the supranuclear region of the enterocytes (Brunser *et al.*, 1970; Mathan *et al.*, 1975a). Mathan *et al.* (1975a) also found occasional dark-staining degenerating epithelial cells in the upper two-thirds of the villus, away from the zone of extrusion.

In order to try to overcome some of the difficulties inherent in the interpretation of jejunal biopsies by different investigators, photographs of the dissecting and light microscopic appearance of seven jejunal biopsies from apparently healthy asymptomatic south Indian subjects with varying degrees of enteropathy were circulated to 42 different investigators in various countries. Respondants were asked to indicate the approximate frequency with which such a biopsy might be seen in apparently healthy people in their area. The results confirmed that both dissecting and light microscopic abnormalities were much more prevalent in apparently healthy subjects living in the tropics than in similar people living in temperate climates (Baker, 1973).

3.3. Intestinal Function

In addition to architectural and histological changes in the intestinal mucosa, there is often some evidence of impaired intestinal function. The test most frequently employed to study small bowel function has been the xylose tolerance test. Decreased xylose absorption has been demonstrated by a number of investigators (Sprinz *et al.*, 1962; Angel *et al.*, 1963; Lindenbaum *et al.*, 1966a,b; Russell *et al.*, 1966; Banwell *et al.*, 1967; Robins *et al.*, 1967; Troncale *et al.*, 1967; Falaiye, 1969; Halsted *et al.*, 1969; Baker and Mathan, 1972; Einstein *et al.*, 1972; Schneider and Viteri, 1972; Klipstein *et al.*, 1972, 1973a, 1976; Thomas *et al.*, 1976).

In some countries a small proportion of apparently normal subjects has been found to have steatorrhea (Robins *et al.*, 1967; Cowan *et al.*, 1971; Cowan, 1972; Troncale *et al.*, 1967; Baker and Mathan, 1972). In south India, even in subjects without steatorrhea, the mean fat excretion is higher than that of healthy control subjects in England, suggesting a widespread mild impairment of fat absorption (Baker and Mathan, 1972).

Malabsorption of vitamin B₁₂ has been found in from 3 to 50% of appar-

ently normal subjects living in the tropics (Lindenbaum *et al.*, 1966a,b; Troncale *et al.*, 1967; Klipstein, 1971; Baker and Mathan, 1972; Klipstein *et al.*, 1972, 1973a,b; Nasr *et al.*, 1976). Increased losses of fecal nitrogen have been reported in some individuals (Nasr *et al.*, 1976; Klipstein *et al.*, 1972, 1973a; R. E. Schneider and F. E. Viteri, personal communication, 1976), and reduced absorption of glycine and glycyl-glycine has been demonstrated by intestinal perfusion studies in apparently healthy southern Indian villagers as compared with English subjects living in England (Hellier *et al.*, 1976).

The nutritional consequences of these abnormalities of absorption have not been fully investigated. R. E. Schneider and F. E. Viteri (personal communication, 1976) determined energy balances in patients with tropical enteropathy and were able to demonstrate a significant energy wastage. If this be the case wherever this lesion is prevalent, then it may have important nutritional consequences, especially for those already existing on a marginal energy intake.

3.4. Pathogenesis

3.4.1. Epidemiology

Human fetuses in India (Baker *et al.*, 1962) and Africa (Stanfield *et al.*, 1965; Cook *et al.*, 1969) have fingerlike villi. The architectural change in the villi must therefore be acquired. From study of postmortem specimens, it has been shown that the lesion appears in the first few months of life and is maximal in older children and adults (Chacko *et al.*, 1969).

Impairment of xylose absorption has also been shown to be prevalent in preschool children in south India (Baker and Mathan, 1972) and Bangladesh (Einstein *et al.*, 1972; Harper, 1972), confirming the early onset of abnormalities in intestinal function. In south India, xylose malabsorption was found to be more prevalent in apparently healthy subjects living in villages than in "hospital control subjects" largely of urban origin (Baker and Mathan, 1972). In Guatemala, the prevalence of xylose malabsorption was highest in males living in rural areas, lower in a comparable group of men serving in the army, and lowest of all in urban middle class students of a military academy (Schneider *et al.*, 1974). In a rural village in Guatemala, the prevalence of enteropathy significantly decreased with the introduction of a protected water supply (R. E. Schneider and F. E. Viteri, personal communication, 1976). Further, when inhabitants of tropical countries migrate to temperate climates, the intestinal lesion improves (Gerson *et al.*, 1971a; Lindenbaum *et al.*, 1972; Klipstein and Falaiye, 1969). There is thus evidence that the lesion of tropical enteropathy in the indigenous population is acquired early in life and is more prevalent in rural populations who, presumably, are more exposed to some dietary or environmental "toxin."

Studies of migrants also support the dietary or environmental toxin theory. When expatriates from temperate climates move to tropical countries, they tend fairly rapidly to develop an intestinal lesion similar to that of the local inhabitants (Keusch *et al.*, 1970; Lindenbaum *et al.*, 1966b; Sheehy *et al.*, 1965, 1968; S. J. Baker and V. I. Mathan, unpublished observations, 1970). Moreover, in Bangladesh, Lindenbaum *et al.* (1966a) have shown that the enteropathy was worse in expatriate Peace Corps volunteers who were exposed to the local environment than in expatriate embassy personnel who led a more sheltered existence. These changes in expatriates tend to regress when the individual returns home (Lindenbaum *et al.*, 1971; Sheehy *et al.*, 1965, 1968).

In Puerto Rico there appears to be a seasonal pattern to the prevalence of tropical enteropathy, with a peak occurring in the month of January (Klipstein *et al.*, 1972; Klipstein and Corcino, 1974). However, these studies were only carried out over a short period of time, and it is therefore not certain that this is an annual occurrence.

All of the above studies strongly suggest that dietary or environmental factors of some sort are responsible for tropical enteropathy, but precisely what these factors are is not clear. A number of different conditions have been described that are prevalent in tropical areas and are known or suspected to be capable of damaging the intestine, including nutritional deficiencies and infectious agents.

3.4.2. Nutritional Factors

A variety of nutritional deficiencies have been described, often on rather insubstantial evidence, as causing morphological changes in the gastrointestinal tract of man (Baker, 1977). However, the abnormalities of tropical enteropathy are seen even in subjects eating a good diet (Baker *et al.*, 1962; Lindenbaum, 1968), and, as noted above, they may occur in well-nourished expatriates shortly after their arrival in the tropics. It therefore seems improbable that dietary deficiencies *per se* account for the lesion.

Klipstein and Corcino (1974) have suggested that seasonal changes in dietary practices such as an increased intake of long-chain unsaturated fatty acids might alter the intestinal microenvironment and favor colonization by enterotoxigenic bacteria (see Section 3.4.4), thus accounting for the seasonal pattern of prevalence of tropical enteropathy seen in Puerto Rico.

3.4.3. Parasites

Parasites such as *Strongyloides stercoralis* (Stemmermann, 1967), *Giardia lamblia* (Yardley *et al.*, 1964), *Capillaria philippinensis* (Whalen *et al.*, 1969), coccidiosis (Brandborg *et al.*, 1970), and cryptosporidiosis (Meisel *et al.*, 1976)

can produce intestinal damage in man sufficient to cause a frank malabsorption syndrome, and it is possible that milder infestations might produce lesser degrees of damage. However, the distribution and prevalence of these parasites are not such as to explain the ubiquitous nature of tropical enteropathy. It has been claimed that hookworm infestation can cause morphological changes in the small intestine (Sheehy *et al.*, 1962a; Tandon *et al.*, 1966a; Chuttani *et al.*, 1967), but unfortunately these studies were done in areas where tropical enteropathy is prevalent, and more critical studies suggest that hookworm plays little or no role in the production of these lesions (Layrisse *et al.*, 1964; Burman *et al.*, 1970). Moreover, the lesion appears in very young children before they have contracted hookworm or other parasitic infestations (Chacko *et al.*, 1969).

3.4.4. Bacteria

Animals raised in a bacteria-free environment have taller villi, shorter crypts, and less cellular infiltration of the lamina propria in the small intestine than their conventionally raised counterparts (Abrams *et al.*, 1963). Several studies of the bacterial flora of the intestine of residents in the tropics, especially those living in rural areas, have shown increased numbers of bacteria in the upper jejunum of at least some subjects (Gorbach *et al.*, 1970; Bhat *et al.*, 1972, 1980; Mata *et al.*, 1972a; Klipstein *et al.*, 1973a). However, it should be noted that subjects in temperate zones who have bacterial overgrowth associated with a stagnant loop syndrome do not display histological abnormalities in their intestinal mucosa (Donaldson, 1965) or at most have a mild patchy lesion (Ament *et al.*, 1972). Furthermore, bacterial contamination of the bowel tends to increase towards the ileum, yet the lesion of tropical enteropathy is, if anything, more marked proximally than distally (Baker *et al.*, 1962; Chacko *et al.*, 1969). It must, however, be pointed out that bacteria that can be aspirated from the gut lumen do not necessarily reflect the total population of intestinal bacteria, since there may be quite a different flora closely adherent to the mucosa (Bhat *et al.*, 1980). Moreover, failure to isolate a significant number or specific type(s) of organism at one point in time does not rule out the possibility of repeated transient bacterial infections of the upper intestine.

Acute infections with specific bacterial pathogens may be followed by a temporary period of malabsorption (Lindenbaum, 1965). Repeated enteric infections may possibly produce a more long-lasting derangement of intestinal structure and function (Gerson *et al.*, 1971a; Lindenbaum, 1973). In south India (Baker and Mathan, 1972) and in Bangladesh (Einstein *et al.*, 1972), a correlation was observed between the number of episodes of diarrhea and the results of xylose absorption tests. In Rhodesia, (Zimbabwe), Thomas *et al.* (1976) have noted that enteropathy is more marked in individuals of lower socioeconomic status and suggest that this is because of their greater exposure to repeated

gastrointestinal infections. On the basis of observations in Puerto Rico, it has been suggested that repeated infections with organisms not usually classified as enteric pathogens but which elaborate an enterotoxin may be responsible for the intestinal damage (Klipstein and Corcino, 1974; Klipstein and Schenk, 1975a; Klipstein, 1979).

Another possible mechanism whereby bacteria can damage the intestine is by deconjugation of bile salts. Apparently healthy Guatemalan children with tropical enteropathy have been found to have deconjugated bile salts in the upper jejunum (Schneider and Viteri, 1974). However, this has not been found in other parts of the tropics where bile salts have been studied (Cassells *et al.*, 1970; Kapadia *et al.*, 1971a; Desai *et al.*, 1972; Bevan *et al.*, 1974).

Finally, Cook (1972) has suggested that acute or chronic systemic bacterial infections may affect intestinal function. The mechanism by which this occurs is not clear, but if, indeed, the enterocyte itself is affected, then it is possible that repeated systemic infections may also play a role in the production of tropical enteropathy.

3.4.5. Viruses

Inhabitants of the developing regions of the world are exposed to a variety of viral agents from the time of birth. Some, such as rotavirus, produce an acute enteritis from which the patient usually recovers with little or no sequelae. However, many other viral agents may be found in the stools. Thus, viral isolates can be obtained from 20% of stool specimens from Guatemalan village children under 3 months of age (Mata *et al.*, 1972b) and in 58% of randomly collected stools from south Indian preschool children (T. J. John, personal communications, 1972). Other viruses, which may not be cultivatable, may be seen by electron microscopic examination of the stools (Mathan *et al.*, 1975b). The role, if any, of these agents in the pathogenesis of tropical enteropathy is unknown.

3.5. Summary and Conclusions

Enteropathy characterized by asymptomatic abnormalities of small intestinal structure and function is widely prevalent in tropical and developing countries. While the intestine may be affected by many insults, there is considerably circumstantial evidence that microbial contamination of the intestine plays an important role in initiating and perpetuating the lesion. This evidence includes the facts that the enteropathy occurs early in life or soon after arrival in the tropics, it is more prevalent in children who have more bouts of diarrhea, it is more prevalent in rural populations, it is more prevalent in expatriates exposed to the local environment and less in those who are less exposed and, finally, it tends to clear up when individuals move to a more sanitised environment. The precise

organisms involved, bacterial, viral, or others, and the way in which they produce damage remain to be discovered. The effect of this lesion on the nutritional status of tropical inhabitants has not been fully elucidated, but it may well play a significant role in those living on already marginal food intakes. One hopes that as levels of hygiene and sanitation improve in the developing world, this condition will become less prevalent.

4. TROPICAL SPRUE

As noted above, the term tropical sprue is arbitrarily defined as a syndrome occurring among residents of, or visitors to, the tropics, usually characterized by diarrhea and other symptoms together with malabsorption of two or more substances.

4.1. Epidemiology

4.1.1. Geographical Distribution

One of the interesting and as yet unexplained facts about the syndrome of tropical sprue is its geographical distribution. It is widely prevalent on the Indian subcontinent (Bahr, 1915; Baker and Mathan, 1968a). It has also been described in many parts of southeast Asia such as Burma (Walters, 1947; Ayrey, 1948), Thailand (Juttijudata *et al.*, 1969; O'Brien and England, 1966, 1971), Vietnam (Sheehy *et al.*, 1965; Miller *et al.*, 1974; Pittman and Pittman, 1976), Hong Kong (Webb, 1956; O'Brien and England, 1971), Malaya, Singapore, Borneo (O'Brien and England, 1971), Indonesia (Sheehy *et al.*, 1965; O'Brien and England, 1971), and the Philippines (Olson and Layne, 1947; Sparberg *et al.*, 1967). It used to be prevalent in China (Begg, 1912), but this reviewer has been unable to find any recent reference to its occurrence there. It is also prevalent in areas of the Caribbean and neighboring countries such as Puerto Rico (Ashford, 1932; Suarez, 1938; Rodriguez-Molina, 1943; Bayless *et al.*, 1968), the Dominican Republic (Klipstein and Falaiye, 1969), Haiti (Klipstein *et al.*, 1966a), Cuba (Lopez *et al.*, 1949), Guatemala (Klipstein and Falaiye, 1969), Costa Rica (Duran, 1924), Venezuela (Beker *et al.*, 1961), and Colombia (Ghitis *et al.*, 1967).

Although tropical sprue is prevalent in many tropical countries, it is alleged to be rare or absent in others, notably Jamaica and the African subcontinent south of the Sahara. It is, of course, always difficult to exclude the presence of a specific disease in a given geographical area unless a definitive search is made for it by someone familiar with the condition. It is even more difficult to exclude what is probably a syndrome that can be mimicked by a number of other conditions.

Gardner (1958) states that tropical sprue does not occur in Jamaica but does not give any reference to support that statement. P. Milner (quoted in Klipstein, 1971), studying megaloblastic anemia in adults in Kingston, Jamaica, was unable to identify any cases of tropical sprue. Further, although other cases of malabsorption have been investigated at the University Hospital of the West Indies, no case of tropical sprue was recognized in the 20-year period from 1950 to 1970 (E. K. Cruikshank, quoted in Ashcroft, 1970).

Foy and Kondi (1971) reviewed reports of malabsorption syndrome in Africa and concluded that there was no good evidence in the literature for the occurrence of tropical sprue in sub-Saharan Africa. Banwell *et al.*, (1964, 1967), Foy and Kondi (1971), and Cook (1974a) specifically looked for tropical sprue, particularly among patients with a malabsorption syndrome and/or megaloblastic anemia, but were unable to document any cases. On the other hand, Falaiye (1970) described nine Nigerian subjects, and Moshal *et al.* (1975) 24 South African subjects who had a malabsorption syndrome compatible with a diagnosis of tropical sprue. Thomas and Clain (1976) described 31 cases seen in Salisbury, Rhodesia (Zimbabwe), over a 15-month period, that seem to closely resemble the syndrome as seen in the Caribbean. These latter authors point out that the prevalence and geographical distribution of the syndrome in Africa have been inadequately studied—an opinion with which this reviewer is in complete agreement.

There have been four reports of a syndrome “resembling tropical sprue” in subjects who have always lived in temperate zones (Cooke *et al.*, 1963; Kendall and Bayley, 1971; Goldstein *et al.*, 1972; Montgomery *et al.*, 1973). In some of these subjects, the condition appears to have started with a gastroenteritislike illness, whereas in others the onset was insidious. Although these cases certainly bear some similarity to the tropical sprue syndrome, the rarity with which they have been described from centers where sophisticated investigation of gastrointestinal disease is common is in marked contrast to the high prevalence in many regions of the tropics, and for the time being, at least, it would seem prudent to keep these separate from the syndrome of tropical sprue and to confine that term to disease developing in residents of, or visitors to, the tropics.

4.1.2. Epidemic Sprue

One of the fascinating features of tropical sprue is the tendency for it to occur in epidemics. The first suggestion of an epidemic is Hillary's account of the disease in Barbados (Hillary, 1759). This apart, most of the recorded epidemics have been in south India, northwest India, northeast India, East Pakistan (now Bangladesh), and Burma (Baker and Mathan, 1968a). Stefanini (1948) was able to study an epidemic that occurred over a 4-year period among Italian prisoners of war in a camp in the Yol-Kangara valley in the Himalayan foothills.

In south India there are unpublished accounts of at least five epidemics from 1929 to 1940 in the records of the Madras State Health Services (Baker and Mathan, 1968a), and within the last 20 years we have studied several other epidemics, details of some of which have been published (Baker *et al.*, 1963; Mathan *et al.*, 1966; Baker and Mathan, 1968a, 1970, 1972; Mathan and Baker, 1968, 1970, 1971). The smallest of these was an epidemic in an isolated household in which 16 out of 27 members were affected (Mathan *et al.*, 1966), and the largest involved an estimated 100,000 individuals with a case fatality rate of around 30% (Baker and Mathan, 1970). Contrary to the findings of Stefanini (1948), there was no clear-cut seasonal pattern.

Most of the epidemics we have studied have had a number of features in common, such as a higher age-specific attack rate in adults, an epidemic curve that peaks in 2–3 months and then tails off over a period of 12 months or more, a clustering of cases in space and time within the village, and, in one case, evidence that in a second epidemic wave, there was a higher attack rate in children under 5 who had not been exposed at the first epidemic 5 years earlier (Mathan and Baker, 1971). In all of the epidemics, many subjects have only a short-lived diarrhea which clears within a few days or weeks without any apparent residual damage, whereas others go on with diarrhea for months or years, with either a continuous course or one punctuated by remissions and relapses, and develop all the features of classical tropical sprue. The epidemiologic features are the same, irrespective of the duration of symptoms, suggesting that the same “agent” is responsible for both the short self-limiting condition and the full-blown syndrome of tropical sprue.

Dean and Jones (1972) and Jones *et al.*, (1972) describe seasonal epidemics of gastroenteritis at an American military base in the Philippines. They postulated a water-borne infectious agent as the cause of the epidemic but were unable to isolate any agent. Usually, the illness cleared up in a few days, but in 22% of patients it lasted more than 3 weeks and was accompanied by xylose malabsorption, decreased mucosal disaccharidases, and mild changes in intestinal biopsy and small bowel X-ray findings. Symptomatic relief was obtained by treatment with tetracycline and folic acid. In the present state of knowledge, it is impossible to know if these epidemics bear any relation to the epidemics of sprue seen in India.

4.1.3. Endemic Sprue

In India (and possibly the Philippines), tropical sprue occurs in both endemic and epidemic forms, but in other countries only the endemic form has been described. There are no good studies of the epidemiology of endemic sprue. It is usually thought of as being a disease of adults, but Ashford (1932) in Puerto Rico found that 10% of his cases were in children under 10, and more recently

well-documented descriptions have been published of the condition in children in south India (Mathan *et al.*, 1969), north India (Mehta *et al.*, 1968), and Puerto Rico (Santiago-Borrero *et al.*, 1970).

In a study of 21,000 people in 25 villages in south India, the age-specific attack rate of chronic diarrhea clinically resembling tropical sprue was found to have very similar pattern to the age-specific attack rate in epidemic sprue; i.e., all age groups were affected, but there was a lower incidence in adolescents (0.6%) and a higher incidence among those over 30 (2.2%) (Baker and Mathan, 1972).

In Puerto Rico, Sheehy *et al.* (1965) found an annual incidence of sprue among North American adults (military personnel and their families) of approximately 8%—a rate which must almost qualify for description as an “epidemic.” Although some children had intermittent diarrhea, none were included in the study.

Little is known about the epidemiology of the tropical sprue in the indigenous populations in the Caribbean region. In both Puerto Rico and Cuba there is some evidence that the incidence has declined (Roderiguez-Molina, 1943; Milanés, 1960; Sheehy *et al.*, 1965). Klipstein and Corcino (1974), studying 27 patients hospitalized with tropical sprue, found a peak onset of symptoms in the winter months of December to February. These authors postulate that this may be related to changes in dietary patterns.

Further clarification of the true geographical distribution and epidemiology of epidemic and endemic forms of tropical sprue must await an understanding of the etiology, or etiologies, of the syndrome and the development of diagnostic and screening tests suitable for population studies.

4.2. Clinical Features

The vast majority of patients with tropical sprue present with a history of chronic diarrhea. This may develop insidiously so that the individual is unable to determine with any accuracy when the condition began. In other subjects, the first symptom may be a well-remembered attack of acute watery diarrhea, sometimes accompanied by mild fever, nausea, and vomiting. Initially this may be diagnosed as gastroenteritis, but, unlike the usual attack of gastroenteritis, the illness persists, the stools become less fluid, and gradually symptoms and signs of secondary deficiencies develop. It is not uncommon for subjects who present in this way to have been associated with others who were similarly affected but in whom the initial illness cleared up without the development of evident malabsorption (O'Brien and England, 1966, 1971; Baker and Mathan, 1968a; Mathan and Baker, 1971). With either mode of presentation the diarrhea may be continuous or remittant. Associated with the diarrhea, there are often other gastrointestinal symptoms such as anorexia, abnormally loud borborygmi, abdominal fullness or

distension, nausea, and vomiting. Occasionally, patients present without preceding gastrointestinal symptoms but with one of the complications of malabsorption such as megaloblastic anemia, and no history of diarrhea can be elicited. Initial symptoms may develop within 5–6 days of entering an epidemic area (Baker and Mathan, 1968a) or may not appear for many years after leaving the tropics (Manson-Bahr, 1957; Mollin and Booth, 1968).

Physical examination in the early stages is usually noncontributory, although loud borborygmi and abdominal distension may be present, and, in thin people, there may be visible small intestinal peristalsis. At any stage of the illness, patients with severe diarrhea may develop dehydration, hyponatraemia, and hypokalaemia. As the condition continues, multiple deficiency states may develop, leading to anemia, dependant edema, ascites, glossitis, angular stomatitis, xerosis conjunctivae, peripheral neuropathy, etc. It is important to note that the prevalence of deficiency states increases with increasing duration of illness, suggesting that they are the result and not the cause of the illness (Stefanini, 1948; Gardner, 1958; O'Brien and England, 1971; Baker and Mathan, 1971). The extent and rate at which these deficiency states develop depend on a number of factors, including the nutritional status of the individual prior to the onset of the illness, the severity of the illness, and the dietary intake during the course of the disease.

Although malabsorption is the major factor in the development of most deficiency states, other factors also play a part. Decreased food intake because of anorexia is common. This anorexia may occur early in the course of the disease (Baker, 1957). As the disease progresses, nutritional deficiencies, particularly of folate and vitamin B₁₂, themselves contribute to the anorexia (Klipstein and Corcino, 1977), forming a vicious cycle. Excessive nutrient losses are obviously important in the fluid and electrolyte disorders; excessive loss of protein (Rubini *et al.*, 1961; Vaish *et al.*, 1965; Jeejeebhoy *et al.*, 1969) and folate (Baker and Mathan, 1971) have also been demonstrated. Finally, decreased synthesis of albumin has been shown to be an important factor in the genesis of hypoalbuminemia in some patients (Jeejeebhoy *et al.*, 1969; Singh *et al.*, 1973).

Examination of the stools usually shows an increase in 24-hr stool volume. Classically, but by no means always, the stools are loose, pale, frothy, and foul smelling. Microscopic examination will usually show fat droplets and at times undigested meat fibers. A careful search should be made for parasites, and if present, their role in the production of the patient's illness must be elucidated by treating the infestation and seeing whether or not the patient's condition improves.

Radiological examination of the gastrointestinal tract is essential to rule out other causes of the malabsorption syndrome. Changes that may be seen in tropical sprue include dilation of the duodenum and small intestine, a coarse mucosal pattern, and alterations in peristaltic activity (Paterson and Baker, 1958). These

abnormalities are more marked with increasing duration and severity of the illness (O'Brien and England, 1971; Baker and Mathan, 1971).

4.3. Absorptive Defects

Although absorption of water and electrolytes (Banwell *et al.*, 1970; Corcino *et al.*, 1973) and many foodstuffs is interfered with, fat, carbohydrate, folic acid, and vitamin B₁₂ have been studied the most.

4.3.1. Fat

The proportion of patients found to have steatorrhea varies in different series. In south India (Baker and Mathan 1968b, 1971), north India (Stefanini, 1948; Misra *et al.*, 1967), Singapore (O'Brien and England, 1971), and Puerto Rico (Caldwell *et al.*, 1965), 90–100% of subjects have had steatorrhea. On the other hand, Elder (1947), Gardner (1956), and Jeejeebhoy *et al.* (1966) record steatorrhea in only 50% to 60% of cases. The reason for these differences is not clear. Elder's study was in wartime, before the advent of jejunal biopsy, and it is possible that some of his subjects were suffering from other conditions. Since the degree of steatorrhea tends to be related both to the duration of symptoms and to the severity of the histological changes (Baker and Mathan, 1971; O'Brien and England, 1971), the patients described by Gardner (1956) and by Jeejeebhoy *et al.* (1966) may have been milder cases or earlier in the course of their disease than those studied by other investigators. The prevalence of steatorrhea will also be affected to some extent by the type of fat in the diet, since medium-chain triglycerides are better absorbed than the longer-chain triglycerides (Cancio and Menendes-Corrada, 1964), and unsaturated fats slightly better than saturated ones (Mehta *et al.*, 1971).

Normal fat absorption consists of a number of steps—the formation of micelles, the secretion and action of pancreatic enzymes, the entry of the products of fat digestion into the enterocyte, the resynthesis of triglyceride within the cell, and its transport out of the cell as chylomicrons. It seems probable that in tropical sprue there may be interference with this process at a number of points. Banwell and Gorbach (1969) suggested that bacteria in the jejunum of patients with tropical sprue might cause deconjugation and dehydroxylation of bile salts in the upper small bowel, thus reducing their concentration below the critical micellar level. However, several groups of investigators have demonstrated that bile salt deconjugation does not occur in the upper intestine (Casells *et al.*, 1970; Kapadia *et al.*, 1971a; Desai *et al.*, 1972; Bevan *et al.*, 1974). On the other hand, Kapadia *et al.* (1971b) found an elevated ratio of glycine to taurine conjugates in the jejunum of sprue patients, suggesting interference with bile salt

reabsorption (McLeod and Wiggins, 1968; Garbutt *et al.*, 1969). This has been confirmed by the finding of excessive loss of labeled bile salts from the body (Kapadia and Baker, 1973) and decreased luminal bile salt concentration in the duodenum (Desai *et al.*, 1972; Bevan *et al.*, 1974). It is probable that these bile salt abnormalities play some part in the pathogenesis of the steatorrhea, but neither the glycine/taurine ratio nor the rate of excretion of bile salts correlates with the degree of steatorrhea, so other factors must also be important.

Exocrine pancreatic secretion following a standard test meal showed a significant reduction in pancreatic function in 24 south Indian patients with tropical sprue as compared with 24 control subjects (Balagopal *et al.*, 1975). However, this study did not enable a differentiation to be made between defective endogenous production of CCK/pancreozymin and an intrinsic defect of pancreatic secretion, nor did it define the precise contribution of pancreatic dysfunction to the fat malabsorption.

In order to throw more light on the relative roles of micelle formation and triglyceride hydrolysis in the steatorrhea of tropical sprue, detailed studies of intraluminal fat digestion will be necessary.

The role of subsequent events in the genesis of the fat malabsorption of tropical sprue has been very inadequately studied. It has been demonstrated *in vitro* that there is both a defect of fatty acid uptake by mucosal cells and a defect of its intracellular conversion to triglyceride, but the mechanisms responsible for this are not clear (Baker and Rao, 1962).

Histochemical studies show an accumulation of lipid in the basement membrane region of the jejunal mucosa in biopsies taken after a 12 to 14-hr fast (Schenk *et al.*, 1965, 1968; Baker and Mathan, 1971). This suggests a holdup in transport of fat enroute from the enterocyte to the lymphatics. Electron microscopy shows that this lipid is, in fact, in a thickened sub-basement-membrane region together with collagen and an amorphous deposit of unknown composition (Baker and Mathan, 1971; Mathan *et al.*, 1975a).

In summary, it is probable that the steatorrhea associated with tropical sprue is multifactorial in origin, but much work remains to be done to fully clarify its pathogenesis.

4.3.2. Carbohydrate

4.3.2a. Glucose. Glucose absorption, as judged by an oral glucose tolerance test, is often impaired in patients with tropical sprue (Fairley, 1936; Stefanini, 1948; Gardner, 1956, 1958; Baker, 1957; Rajan *et al.*, 1961). The precise mechanism of this malabsorption is unknown. Although glucose absorption has been used as a clinical test of intestinal function, the fairly large overlap with normal subjects has caused it to fall into disrepute as a diagnostic test (Gardner, 1956; Rajan *et al.*, 1961).

4.3.2b. Xylose. Xylose absorption has been widely used as a test of intestinal function. The vast majority of investigators have found a high prevalence (73–100%) of xylose malabsorption in subjects with tropical sprue (Klipstein, 1970). The one exception are the patients described by Tandon *et al.* (1966b) of whom only 18% had xylose malabsorption. The reasons for the very low prevalence in this study are not clear, but this finding must raise questions about the nature of their cases.

In those regions where tropical enteropathy is common, there will be a high prevalence of xylose malabsorption in the general population, and this must be taken into account in studying the prevalence of xylose malabsorption in patients with sprue. In south India, although there is some overlap, the frequency distribution of xylose excretion in subjects with sprue is different from that of subjects with tropical enteropathy, indicating two distinct populations (Baker and Mathan, 1972). This overlap, however, limits the diagnostic usefulness of the xylose test.

The pathogenesis of the xylose malabsorption is not understood. It is usually assumed to reflect enterocyte damage, but much more work remains to be done to elucidate the precise nature of the defect involved.

4.3.2c. Disaccharides. Several groups of workers have demonstrated that brush border disaccharidase activities are reduced in patients with tropical sprue (Santini *et al.*, 1960; Bayless *et al.*, 1964; Gray *et al.*, 1968; Sheehy and Anderson, 1965; Swaminathan *et al.*, 1970; Corcino *et al.*, 1976).

In view of the fact that the number of mature enterocytes is decreased, and the brush border of individual enterocytes may be sparse and deformed (see Section 4.4), it is to be expected that enzyme activity expressed per milligram of protein or per milligram of tissue will be reduced. It is also not surprising that there is some correlation between the decrease in disaccharidase activity and the degree of histological abnormality (Swaminathan *et al.*, 1970).

4.3.3. Folic Acid

There is a high prevalence of folate deficiency in subjects with tropical sprue (Klipstein, 1972), and it is reasonable to assume that folate malabsorption plays a significant role in the pathogenesis of this deficiency. Surprisingly, tests of folate absorption have provided conflicting results. The situation is further confused by the wide variety of folate absorption tests employed by different investigators (Baker, 1976). Some investigators employing pharmacological doses have demonstrated decreased absorption of pteroylmonoglutamic acid (PGA) in patients with tropical sprue (Butterworth *et al.*, 1957; Chanarin *et al.*, 1958; Jeejeebhoy *et al.*, 1966; Ghitis *et al.*, 1967; Klipstein *et al.*, 1968; Klipstein, 1971). However, in one study, peak serum concentrations following a large

oral dose of PGA were no different in patients with tropical sprue than in control subjects (Jeejeebhoy *et al.*, 1968).

When physiological doses of tritiated PGA are employed in absorption studies, it is more difficult to demonstrate defective absorption. Thus, Jeejeebhoy *et al.* (1967), using a 320- μ g dose of PGA, could demonstrate defective absorption in only eight out of 17 patients. In south India, using a 200- μ g dose, only nine of 69 patients with tropical sprue had demonstrable malabsorption of PGA (Baker and Mathan, 1971), and using a 25- μ g dose Klipstein (1969) found normal absorption in all of three patients with tropical sprue and folate deficiency. Smith *et al.* (1970) demonstrated that PGA absorption in the rat is stimulated by the presence of glucose. In man, Gerson *et al.* (1971b) and Corcino *et al.* (1975, 1976) studied PGA absorption from a segment of upper jejunum by the perfusion technique. When the PGA was in glucose- or galactose-free medium, absorption of folate in patients with sprue was not significantly different from normal; however, in the presence of glucose or galactose, the stimulation of PGA absorption was significantly lower in the sprue patients. The biochemical explanation for this phenomenon is not clear, but is presumably a reflection of mucosal cell damage and may be related to defective absorption of glucose.

The comparative rarity of nutritionally significant PGA malabsorption is further evidenced by the fact that many patients with tropical sprue with folate deficiency megaloblastic anemia will respond to small oral supplements of PGA (Sheehy *et al.*, 1961; Baker and Mathan, 1971). This fact is of considerable practical importance, since it means that it is perfectly feasible to treat the frequently occurring folate deficiency of tropical sprue by the administration of oral folic acid.

The absorption of folate polyglutamates (the major naturally occurring form in foods) is more difficult to study. Hematological methods employing the double reticulocyte response suggested that food folate may be less well absorbed than PGA (Baker, 1966). Other workers, following the rise in serum folate concentration after an oral dose of polyglutamate, demonstrated defective polyglutamate absorption in 50–100% of cases (Jeejeebhoy *et al.*, 1968; Hoffbrand *et al.*, 1969; Bernstein *et al.*, 1970), and Corcino *et al.* (1976), using [^{14} C]hexaglutamate in an intestinal perfusion system, noted improved absorption in six patients with tropical sprue following treatment.

The pathogenesis of folate polyglutamate malabsorption is not clear. It is known that, during absorption, polyglutamates are largely converted to the monoglutamate form (Butterworth *et al.*, 1969). This conversion is catalyzed by the enzyme γ -glutamyl-peptidase (conjugase). Although conjugase is present to some extent in the intestinal lumen, the bulk of it is associated with intracellular lysozymes. Moreover, luminal pH does not favor conjugase activity, and it is probable that the breakdown of polyglutamates occurs mainly within the entero-

cyte. Curiously, measurements of intraluminal (Klipstein, 1967a) and intracellular (Hoffbrand *et al.*, 1969; Corcino *et al.*, 1976) conjugase activity have not shown a significant decrease in the majority of folate-deficient sprue patients. Although the number of mature enterocytes is decreased in patients with tropical sprue, and therefore the total conjugase activity per unit weight of tissue might be expected to be reduced, there is also an increase in number of lysozymes per cell (see Section 4.4.3), which may compensate for the decrease in number of mature cells. In this respect, it would be of interest to compare the relative concentration of conjugase with that of other lysosomal enzymes. Perhaps the normal rate-limiting step is the entry of polyglutamates into the enterocyte—any reduction in the number of mature enterocytes would then result in a reduction of polyglutamate absorption.

4.3.4. Vitamin B₁₂

Vitamin B₁₂ malabsorption has been reported to be present in from 17% (Meyer *et al.*, 1953) to 100% (Klipstein *et al.*, 1966a) of patients with tropical sprue. In south India we have observed significant differences in the prevalence of vitamin B₁₂ malabsorption between patients with endemic and epidemic sprue and among patients from different epidemics, although the explanation for these differences is obscure (Baker and Mathan, 1971).

The pathogenesis of the vitamin B₁₂ absorptive defect is probably multifactorial. It has long been known that patients with tropical sprue frequently have hypochlorhydria or achlorhydria (Fairley, 1930). This is associated with a gastritis which may range from a simple increase in cellularity of the lamina propria to a severe atrophic gastritis or gastric atrophy (Floch *et al.*, 1963; Vaish *et al.*, 1965; Baker, 1967). In 4–7% of cases, intrinsic factor is completely absent, and in a further 7%, it is within the range found in patients with pernicious anemia (Wheby and Bayless, 1968; Baker, 1972). In such cases, if there is no other defect of vitamin B₁₂ absorption, repeating the absorption test with exogenous intrinsic factor will normalize the result (Baker and Rao, 1962). In south India, this only occurred in 10% (3 out of 30) of subjects when the test was repeated with intrinsic factor within 10 days of the original test. In the remainder of cases with vitamin B₁₂ malabsorption there must be some other explanation for the defect.

In some patients with tropical sprue vitamin B₁₂ absorption may be normalized by the use of broad-spectrum antibiotics (Mollin *et al.*, 1957; Klipstein, 1964a,b; Guerra *et al.*, 1965; Gorbach *et al.*, 1970; O'Brien and England, 1971; Mollin and Booth, 1971; Baker, 1972; Tomkins *et al.*, 1978). In a longitudinal study of 72 south Indian patients with vitamin B₁₂ malabsorption, 36 were given antibiotics, and 36 were not—there was no differences in the numbers in the two groups in whom vitamin B₁₂ absorption was normalized. However, in those

given antibiotics, vitamin B₁₂ absorption often began to improve within 2 to 5 days (Baker, 1967, 1972; Baker and Mathan, 1971).

A similar early response of some patients to antibiotics has been found by Tomkins *et al.* (1978). This rapid response to antibiotics suggests that, in these cases, bacteria must play a role in the pathogenesis of the vitamin B₁₂ malabsorption, as has been shown to be the case in the stagnant bowel syndrome (Donaldson, 1962). However, using classical microbiological methods, no quantitative or qualitative difference could be found in luminal microbial flora of jejunum or ileum of patients with and without vitamin B₁₂ malabsorption (Bhat *et al.*, 1972). These studies have subsequently been extended to a total of 46 vitamin B₁₂ malabsorbers and 40 with normal absorption with similar results (Albert *et al.*, 1982), so it is still not possible to define which bacteria are responsible. Furthermore, studies to try and delineate the mechanism by which bacteria interfere with vitamin B₁₂ absorption have been unsuccessful. It has been shown that the intrinsic factor–vitamin B₁₂ complex is not taken up or degraded by bacteria and that it reaches the ileum in normal amounts (Kapadia *et al.*, 1975). This suggests that either the intraluminal bacteria produce a “toxin” that damages the receptor or in some other way interferes with the binding of the vitamin B₁₂–intrinsic factor complex to the receptor, or the bacteria responsible for the vitamin B₁₂ malabsorption are closely adherent to the mucosal surface and are not sampled by aspiration of luminal contents (Bhat *et al.*, 1980). Carmel *et al.* (1968), in one Puerto Rican patient who may have had tropical sprue, showed defective *in vitro* uptake of vitamin B₁₂–intrinsic factor complex by ileal mucosa, but the mechanism of the defect was not explored. There is obviously a need for further studies of the ileal receptor in patients with sprue to determine the prevalence and pathogenesis of such a lesion.

In those subjects in whom the vitamin B₁₂ malabsorption is not improved by antibiotics, it must be assumed that either bacteria play no role in the pathogenesis of the malabsorption or that the bacteria responsible are not susceptible to the antibiotics employed.

4.4. Pathology

4.4.1. Villus Architecture

Even though alterations in villous architecture are common among apparently healthy asymptomatic subjects living in the tropics (see Section 3.2), the abnormalities in subjects with tropical sprue are more severe. However, a “flat mucosa,” as seen frequently in cases of celiac disease, is an uncommon occurrence (Baker *et al.*, 1962; England, 1968; Klipstein, 1968; Desai *et al.*, 1969; Brunser *et al.*, 1970; Baker and Mathan, 1971, 1972).

4.4.2. Light Microscopy

Light microscopy reveals changes that vary from "mild," with a slight increase in the depth of the crypts and an increase in the cellular infiltration of the lamina propria and epithelium, to "severe," with reduction in the overall mucosal thickness, crypts extending the full depth of the mucosa, increased numbers of enterocyte mitoses, a cuboidal or pseudostratified appearance of the surface epithelium, and a marked cellular infiltration of the lamina propria and epithelium (Chacko *et al.*, 1961; Baker *et al.*, 1962; England, 1968; Schenk *et al.*, 1965, 1968; Klipstein, 1968; Brunser *et al.*, 1970; Bayless *et al.*, 1971; Baker and Mathan, 1971). The nuclei of the crypt enterocytes may be macrocytic (Veeger *et al.*, 1965; Swanson *et al.*, 1966; Wheby *et al.*, 1968). Although vitamin B₁₂ and/or folate deficiency often plays some role in the production of this macrocytosis, it has been observed to persist long after adequate therapy has corrected any vitamin B₁₂ or folate deficiency (Wheby *et al.*, 1968), suggesting the presence of some other unknown factor interfering with DNA synthesis in the developing enterocytes. Schenk *et al.* (1965, 1968) describe thickening of the basement membrane region with accumulation of lipid-staining material in this area and suggest that this is characteristic of tropical sprue. We have also seen this in 12 out of 18 subjects with tropical sprue (Baker and Mathan, 1971) but doubt its specificity, since it has also been seen, although to a lesser degree, in asymptomatic control subjects (Mathan *et al.*, 1975a).

Most investigators have shown some correlation between the severity of histological abnormalities and the degree of malabsorption (England and O'Brien, 1966; Kent and Lindenbaum, 1967; Baker and Mathan, 1971; O'Brien and England, 1971; Klipstein, 1971). There is also evidence, at least among expatriates who develop tropical sprue, that the severity of the histological lesion tends to increase with increasing duration of symptoms (England and O'Brien, 1966; O'Brien and England, 1971).

Ileal mucosal biopsies have been studied relatively infrequently. Baker *et al.* (1962) and Wheby *et al.* (1971) found that the histological lesion was generally similar to that seen in the jejunum. England (1968), on the other hand, found that the histological lesion was usually less marked in the ileum. Baker *et al.* (1962) and England (1968) could find no correlation between ileal structure and vitamin B₁₂ absorption; however, with larger numbers (27), Wheby *et al.* (1971) did find that the more severe ileal histological abnormalities were related more frequently with the presence of vitamin B₁₂ malabsorption and vice versa.

4.4.3. Electron Microscopy

Electron microscopy studies have been reported by three groups of investigators (Hartman *et al.*, 1960; Brunser *et al.*, 1970; Mathan *et al.*, 1975a). One of

these studies was technically unsatisfactory because of poor fixation (Hartman *et al.*, 1960). The other two confirmed the findings on light microscopy and, in addition, found an increase in cytoplasmic lysosomes, shortening and grouping of the microvilli, and deposition of a dense layer below the basement membrane containing collagen, an amorphous material, and fat droplets (Brunser *et al.*, 1970; Baker and Mathan, 1971; Mathan *et al.*, 1975a). The studies in India showed other cellular changes ranging from minor ones, such as dilatation of the rough endoplasmic reticulum and swelling of the mitochondria, to grossly degenerating cells. These were of two types: electron-dense shrunken cells with pyknotic nuclei, increased lysosomes, dilated rough endoplasmic reticulum, and swollen degenerating mitochondria and cells more electron translucent than surrounding cells, with abnormal nuclei, swollen mitochondria, dilated rough endoplasmic reticulum, and distorted microvilli (Mathan *et al.*, 1975a). These dying cells in the crypts and along the sides of the villi strongly suggest that the cells are dying prematurely, presumably damaged by the etiologic agent that causes tropical sprue.

4.5. Therapy

4.5.1. Spontaneous Remission

Unfortunately, there has been insufficient attention paid to the natural history of the sprue syndrome in different parts of the tropics. Spontaneous remission and apparent cure are commonly seen in individuals affected with epidemic sprue in south India (Baker and Mathan, 1968a; Mathan and Baker, 1970). This tendency to remission is less the longer symptoms have been present. Thus, in the first year, 74% remitted spontaneously, whereas of those who had symptoms for more than a year only 8% remitted spontaneously (Mathan and Baker, 1970). A similar tendency to remission has been noted by a number of observers when subjects are hospitalized (Stefanini, 1948; Baker, 1957; Jeejeebhoy *et al.*, 1968; Chuttani *et al.*, 1968a; Gorbach *et al.*, 1970; Baker and Mathan, 1971; O'Brien and England, 1971). This is often ascribed to "rest" or "hospital diet" but may in fact just be the natural course of the syndrome whether the patient is hospitalized or not. It is of interest that although spontaneous remissions have been described from the Caribbean (Gardner, 1958), it is considered to occur only in "mild" cases (Bayless *et al.*, 1968). This would seem to be at least partly related to the fact that tropical sprue in the Caribbean has long been considered a hematological disorder, and a megaloblastic anemia has been part of the diagnostic criteria. Obviously, subjects with a megaloblastic anemia need to be treated with an appropriate hematinic, and it has therefore not been possible to have a control group that did not receive folic acid or vitamin B₁₂.

4.5.2. Correction of Deficiencies

Correction of deficiency states is obviously important and may at times be life saving, particularly when there are gross fluid and electrolyte disorders (Baker and Mathan, 1971) or severe anemia. Treatment of the anemia used to be dietary, giving yeast, liver, or liver extract (Suarez, 1931; Rhoads and Miller, 1934; Fairley, 1934; Walters, 1947); however, with the isolation of folic acid and vitamin B₁₂, these agents have replaced the dietary management.

4.5.3. Folic Acid

Spies *et al.* (1946) in Cuba were the first to employ folic acid in the treatment of the megaloblastic anemia of tropical sprue. As well as producing a prompt hematological response, it produced a marked decrease in stool volume and a reduction in diarrhea which occurred within 1 to 2 weeks in all except two of nine subjects studied. As this was before the introduction of jejunal biopsy techniques, morphological changes in the intestinal mucosa were not studied. Swanson *et al.* (1966) in Puerto Rico treated seven patients with folic acid. The jejunal biopsy appearances improved in all, but in six out of seven abnormalities persisted. These authors suggest that the folate administration had cured the folate deficiency in the gut but that the basic lesion of sprue persisted. Similar improvement but not cure of the mucosal lesion was seen in the majority of patients treated with folic acid by Sheehy *et al.* (1962b) in Puerto Rico, Klipstein *et al.* (1969) in Haiti, and O'Brien and England (1966, 1971) in Singapore. On the other hand, in Puerto Rico, Rodriguez-Molina *et al.* (1960) and in northern India, Chuttani *et al.* (1968a) were unable to demonstrate any beneficial effect of folate on gut function even though the hematological status was improved. Similarly, in south India, studies of patients with both endemic and epidemic sprue have shown no difference in the remission rate of 57 patients just observed in a metabolic ward and 55 given folic acid (Baker and Mathan, 1971). However, in four patients given folic acid, the stool volume and degree of steatorrhea decreased within a few days, suggesting that in these cases, the folic acid might have been responsible for the improvement.

4.5.4. Vitamin B₁₂

Spies and Suarez (1948), in Puerto Rico, treated four patients with tropical sprue with 10–25 µg of vitamin B₁₂ by injection, and in each case the hematological response was accompanied by a decrease in diarrhea. Swanson *et al.* (1966), also in Puerto Rico, found that injections of 30 µg of vitamin B₁₂, daily for 6 days and then weekly, produced improvement in the jejunal biopsy appearances in three of seven patients with tropical sprue. In patients studied in

London with documented vitamin B₁₂ deficiency resulting from chronic tropical sprue, the correction of the deficiency sometimes appeared to result not only in a hematological response but also in an improvement in intestinal structure and function (Booth *et al.*, 1968; Mollin and Booth, 1971). In south India, patients with vitamin B₁₂ deficiency megaloblastic anemia were treated with vitamin B₁₂, 1 µg/day by injection. A good hematological response was obtained, but there was no detectable effect on intestinal structure or function over and above the similar effect noted in subjects with sprue just given a hospital diet (Baker and Mathan, 1971).

4.5.5. Antimicrobials

The first use of an antimicrobial agent in tropical sprue was by Rogers (1938) who treated a patient suffering from bronchitis with sulfamidochrysoidine (Prontosii®) and noted a coincident symptomatic improvement in his gastrointestinal symptoms. During the 1940s in India sulfaguanidine was found to alleviate the diarrhea of sprue, particularly when given early in the clinical course (Chauduri and Rai Chauduri, 1944; Keele and Bound, 1946; Elder, 1947; Walters, 1947; Stefanini, 1948). Maldonado *et al.* (1969) treated 16 Puerto Rican patients with succinylsulfathiazole or sulfaguanidine for a 6-month period. There was symptomatic improvement in all patients within the first week, and after 6 months, there was marked improvement in intestinal function and histology, although some histological abnormalities persisted. Unfortunately, no controls were used, so it is not possible to be certain that the improvement noted was caused by the sulfonamides.

The use of broad-spectrum antibiotics was introduced by French *et al.* (1956) who studied seven British servicemen repatriated from Hong Kong and Malaya. These workers showed that following the sequential administration of chlortetracycline, chloramphenicol, and succinylsulfathiazole given in various orders, there was rapid clinical improvement and normalization of fat absorption. Although there were no control subjects, there was no significant improvement in the 2- to 4-week period of hospitalization before the start of the chemotherapy, and it seems reasonable to assume that the antibiotics played at least some part in producing the improvement. O'Brien and England (1966, 1971) treated 16 expatriate patients in Singapore with a similar type of regimen. Two of their patients had been ill for less than 3 months and responded rapidly. The other 14 had been ill for 3 months or longer and, in contradistinction to the patients of French *et al.* (1956), "symptomatic improvement was very slow," but there was considerable improvement in intestinal function and structure over the ensuing 1 to 3 months. It must be emphasized that such a slow response is very difficult to interpret because of the marked propensity for patients to undergo spontaneous remission (Baker and Mathan, 1971). In a further group of patients, O'Brien and

England (1971) felt that results of treatment were better when both antimicrobials and folic acid were employed, but these studies were also uncontrolled.

Workers in the Caribbean have also reported favorably on the use of broad-spectrum antibiotics given either for a relatively short period or continuously for up to 6 months (Sheehy and Perez-Santiago, 1961; Guerra *et al.*, 1965; Klipstein *et al.*, 1966a,b, 1969; Horta *et al.*, 1971). However, it must again be noted that in none of these studies were there any controls. Moreover, longer-term follow-up (5 years or more) of a group of 17 patients who had been treated with antibiotics for at least 6 months showed persistence or return of abnormalities of intestinal structure and function in a considerable proportion of patients (steatorrhea in three, abnormal xylose in eight, vitamin B₁₂ malabsorption in 13, and abnormal jejunal biopsies in eight) (Rickles *et al.*, 1972). Tomkins *et al.* (1975), in London, showed a prompt (within 2 weeks) improvement of intestinal structure and function following a course of tetracycline in four of five patients with acute tropical sprue. In patients with chronic tropical sprue in London (Booth *et al.*, 1968; Mollin and Booth, 1971), the hematological and gastrointestinal abnormalities were "invariably restored to normal" by treatment with folic acid, vitamin B₁₂ where necessary, and antibiotics. It should be noted again that there were no controls, and in contradistinction to acute tropical sprue, return to normality often occurred only a number of months after completion of the antibiotic therapy.

In Bombay, Jeejeebhoy *et al.* (1966) obtained marked improvement in absorption tests in the majority of 22 patients with tropical sprue treated with tetracycline for 2 weeks. Gorbach *et al.* (1970), in Calcutta, obtained a marked improvement in steatorrhea and vitamin B₁₂ absorption in four out of five patients with sprue within a few days of starting tetracycline therapy. Tandon *et al.* (1974), in Dehli, found improvement in 14 patients treated with a high-protein diet and tetracycline, nine of whom were also given prednisolone; however, histological abnormalities persisted in the jejunum.

On the other hand, Chuttani *et al.* (1968a), also in Dehli, found no response in 73% of subjects treated with sulfonamides, chloramphenicol, or oral streptomycin. Unfortunately, these authors give no breakdown of responses for the three different antibacterial regimens, so it is difficult to assess the significance of their findings. In patients studied in Vellore, antimicrobial therapy also has been less successful. Of 47 patients treated with antimicrobial agents (a sequential combination of tetracycline, chloramphenicol, and succinylsulfathiazole or straight tetracycline) for 2 to 4 weeks, 24 showed no change in steatorrhea; in nine there was improvement within 5 days following the commencement of therapy, whereas in the others the improvement was more gradual, over 10 days to a number of weeks. There was no difference in the folate and vitamin B₁₂ status of those who improved and those who did not. This proportion of patients

showing improvement (23/47) is no better than that in a group of 57 patients who were not given any specific therapy other than bed rest and a standard hospital diet, of whom 29 improved (Baker and Mathan, 1971).

4.6. Etiology and Pathogenesis

4.6.1. Introduction

It is evident that the syndrome of tropical sprue is produced by functional and structural abnormalities of the intestine resulting from the action of one or more unknown damaging “agents.”

It has been suggested that tropical sprue is the tip of the iceberg of tropical enteropathy (Klipstein, 1967b). Certainly from the clinical and pathological point of view the two conditions seem to grade imperceptibly into each other. However, there are distinct epidemiological differences. Tropical enteropathy is prevalent in areas where sprue does not occur or is comparatively rare, such as Iran (Nasr *et al.*, 1976) and Africa (Cook, 1974a). Also, there are villages in India where tropical enteropathy is prevalent but tropical sprue is absent (Baker and Mathan, 1972). Tropical enteropathy is very common in the first year of life (Chacko *et al.*, 1969), whereas tropical sprue is rare in children and has never been described in the first year of life. Finally, tropical enteropathy improves when subjects return to temperate zones (Sheehy *et al.*, 1968), whereas sprue may develop years after leaving the tropics. We therefore believe that these are two independent conditions of differing pathogenesis. Field studies in south India have shown that not every agent producing intestinal damage results in the sprue syndrome (Mathan and Baker, 1968); there is therefore at least some specificity in the unknown initiating agent or agents. It must also be borne in mind that the effects of any initiating agent may be modified by the interplay of various aggravating or perpetuating factors (Baker and Mathan, 1968a).

Any comprehensive explanation of the sprue syndrome must account, *inter alia*, for a number of distinctive aspects of the condition such as:

1. Its geographical distribution.
2. Its occurrence in epidemic form.
3. The long latent period which may sometimes occur between leaving the tropics and developing the disease.
4. The pattern of therapeutic responses to folic acid, vitamin B₁₂, and antibiotics.

Possible etiological factors are nutritional deficiencies, dietary factors, or infectious agents (parasites, algae, fungi, bacteria, mycoplasma, or viruses).

4.6.2. Nutritional Deficiency

Because of the frequent occurrence of deficiency states in patients with advanced tropical sprue, it is perhaps inevitable that deficiencies should be postulated as one cause of this syndrome (Walters, 1947; Stefanini, 1948; Ayrey, 1948). A deficiency state *per se* will not explain the geographical distribution of the condition, but it could perhaps be combined with some other factor with a limited geographical distribution. Although it might be thought that the occurrence of epidemics precludes a deficiency state, it must be recalled that epidemics of deficiency disease are well documented, e.g., pellagra (Goldberger *et al.*, 1920). However, it is hard to visualize how a deficiency state could explain the very long latent period that may occasionally occur.

4.6.2a. Folate. The sometimes dramatic response of patients with sprue to folic acid has led to the postulate that the syndrome may be caused by dietary folate deficiency (Spies *et al.*, 1946). Although many patients with tropical sprue are folate deficient, the prevalence of folate deficiency increases with increasing duration of symptoms (Baker and Mathan, 1971; O'Brien and England, 1971), suggesting that it is a result and not the cause of the syndrome. Moreover, subjects with folate deficiency megaloblastic anemia in Nairobi (Foy and Kondi, 1971) and in south India, where tropical sprue is highly prevalent (Baker and Mathan, 1971), do not develop the syndrome of tropical sprue. Furthermore, dietary deficiency of folate has usually been reported as producing no change in the appearance of the jejunal mucosa (Herbert, 1962; Gough *et al.*, 1963; Forshaw *et al.*, 1964; Halstead *et al.*, 1973) or, in the presence of alcoholism, minor changes (Bianchi, 1970; Hermos *et al.*, 1972). It must be concluded, therefore, that there is no evidence that folate deficiency is the cause of tropical sprue but that folate deficiency is common and frequently contributes to the symptom complex, most notably to the anemia but also at times to the gastrointestinal dysfunction. The greater the contribution to the gastrointestinal lesion, the more dramatic will be the response to folate therapy in improving gastrointestinal function. For some as yet unexplained reason folate deficiency seems to play a smaller role in the gastrointestinal lesion of patients in India than in patients from other areas (see Section 4.5.3).

4.6.2b. Vitamin B₁₂. Although the administration of vitamin B₁₂ to patients with sprue may, at times, improve not only the hematological status but also in some cases the gastrointestinal function (see Section 4.5.4), vitamin B₁₂ deficiency in nonvegetarian subjects usually only develops months or years after the onset of the disease (O'Brien and England, 1971; Mollin and Booth, 1971). Vitamin B₁₂ deficiency may therefore be excluded as a primary cause of the

syndrome, although, like folate deficiency, it may at times be an aggravating or perpetuating factor.

4.6.2c. Protein. The administration of a protein-deficient diet to animals has produced histological changes in the jejunal mucosa in some studies (Platt *et al.*, 1964; Deo and Ramalingaswami, 1965; Mehta *et al.*, 1979) but not in others (Svoboda *et al.*, 1966; Tandon *et al.*, 1969). These differences may be related to the different species employed and to differing degrees of protein restriction (Baker, 1977). The applicability of these studies to the human situation is very doubtful, since the amounts of protein used were much lower than those habitually consumed in areas where sprue is endemic. Nevertheless, it has been suggested by several workers that protein deficiency may produce changes in intestinal structure and function in adults and be a cause of the tropical sprue syndrome (Mayoral *et al.*, 1967, 1968, 1972; Chuttani *et al.*, 1968b; Tandon *et al.*, 1968; Duque *et al.*, 1975). Unfortunately, all of these studies were conducted in areas where tropical sprue and other forms of intestinal disease are prevalent, and it is impossible to be sure whether the hypoproteinemia caused the gut disease or vice versa.

In patients with tropical sprue, the prevalence and severity of hypoproteinemia increases with increasing duration of the illness (Baker and Mathan, 1971; O'Brien and England, 1971), suggesting that the hypoproteinemia is a result of the disease. Patients living in a temperate climate who have hypoproteinemia do not have mucosal abnormalities (Gough *et al.*, 1963; Jensen *et al.*, 1966; Marin *et al.*, 1969), and three Zambian adults with severe hypoproteinemia were shown to have normal intestinal structure and function (Cook, 1974b). It must be concluded that there is inadequate evidence to consider deficient protein intake as a cause of tropical sprue, although at times, like folate and vitamin B₁₂ deficiency, protein deficiency may act as an aggravating or perpetuating factor.

4.6.3. Dietary Factors

French (1955) suggested that consumption of rancid fats might be a cause of tropical sprue. However, there is no evidence to support this hypothesis, and it does not explain why it occurs in some parts of the tropics and not in others. Moreover, it is hard to see how the ingestion of a dietary toxin can explain the phenomenon of the long latent period.

Klipstein and Corcino (1974) noted in Puerto Rico that the peak seasonal incidence of tropical sprue coincided with the time of maximum consumption of pork and pork fat. Mickelson and Klipstein (1975) postulate that the long-chain unsaturated fatty acids, particularly linoleic, have an inhibitory effect on the

gram-positive intestinal bacteria, allowing overgrowth of coliforms which, in turn, produce enterotoxins which cause disease (see Section 4.6.4). This is an interesting hypothesis which needs to be further explored. It would not, however, seem to be relevant in south India where the dietary fat intake is very low and where there is no clear-cut seasonal pattern.

Although gluten-sensitive enteropathy occurs in regions of the tropics where wheat is eaten, it seems to be of low prevalence (Misra *et al.*, 1966; Walia *et al.*, 1966; Nelson, 1973). Bayless (1964) found some improvement in the intestinal lesion of some patients with sprue when they were put on a gluten-free diet, but this was probably a nonspecific effect. Subjects with tropical sprue do not have gliadin antibodies in their serum (Heiner *et al.*, 1964; Bayless *et al.*, 1967; Menéndez-Corrada and Belaval, 1968), and tropical sprue is prevalent in non-wheat-eating populations (Baker *et al.*, 1963). It therefore seems unlikely that sensitivity to gluten plays any significant role in the pathogenesis of the syndrome.

4.6.4. Infectious Agents

An infectious agent has long been postulated as the cause of tropical sprue (Grant, 1854; Galloway, 1905a,b; Begg, 1912). Such a hypothesis could explain the geographical distribution of the disease, the occurrence of epidemics, and, conceivably, the long latent period.

4.6.4a. Parasites. In the past, certain parasitic diseases of the intestine have doubtless been labeled "tropical sprue" (e.g., giardiasis, strongyloidiasis). However, patients whose malabsorption is caused by these organisms will respond to appropriate therapy and do not come within the definition of the tropical sprue syndrome. If the patient's condition does not improve after eradication of the parasite, then it can be assumed that there is some complicating factor or that the disease is not caused by that parasite.

4.6.4b. Algae. There is one report, which has appeared only in abstract form, suggesting that the alga *Prototheca portoricensis* might be the cause of tropical sprue (Bernstein *et al.*, 1973). However, this organism has never been isolated from patients with sprue or visualized in biopsy specimens (Bhat *et al.*, 1972; Klipstein and Schenk, 1975b; Corcino, 1975) even though it has been looked for and is easy to culture. This hypothesis must therefore be abandoned.

4.6.4c. Fungi. Bahr (1915) and Ashford (1931) postulated that sprue was an infection caused by *Monilia ablicans*, but subsequent studies specifically looking for fungi have failed to confirm this suggestion (Milanes *et al.*, 1946; Bhat *et al.*, 1972).

4.6.4d. Bacteria. Bacteria may cause intestinal disease either by invasion of the mucosa (e.g., Whipple's disease) or by the production of enterotoxins. Light and electron microscopic studies of jejunal biopsies from patients with sprue have consistently failed to reveal any evidence of bacteria in the mucosa, so that bacterial invasion as a cause of the syndrome can be excluded (Hartman *et al.*, 1960; Chacko *et al.*, 1961; Baker *et al.*, 1962; Swanson *et al.*, 1966; England, 1968; Klipstein, 1968; Wheby *et al.*, 1968; Brunser *et al.*, 1970; Mathan *et al.*, 1975a).

Many investigators have unsuccessfully cultured stools and/or luminal aspirates for classical bacterial pathogens in patients with sprue (Nadel and Gardner, 1956; Desai *et al.*, 1966; Klipstein and Samloff, 1966; O'Brien, 1968; Baker and Mathan, 1971; Gorbach *et al.*, 1969, 1970; Bhat *et al.*, 1972). Similar negative results were obtained from culture of jejunal biopsy material (Bhat *et al.*, 1980). Even in epidemics of sprue, known pathogenic organisms have not been isolated with any greater frequency than in control subjects (Baker *et al.*, 1963; Mathan *et al.*, 1966; Baker and Mathan, 1971).

Although classical pathogenic organisms have not been incriminated, a number of investigators in different parts of the world have demonstrated the presence of increased numbers of luminal bacteria in the small intestine in many subjects with tropical sprue (Milanes *et al.*, 1946; Nadel and Gardner, 1956; Klipstein *et al.*, 1966a, 1973b; Lahri *et al.*, 1970; Gorbach *et al.*, 1969, 1970; Bhat *et al.*, 1972; Tomkins *et al.*, 1975; Applebaum *et al.*, 1980). In a number of these studies, at least some of the control subjects also had an abnormal bacterial flora, and in several studies, no difference could be detected between the patients with sprue and the control subjects with respect to the types of organism and their prevalence and distribution within the gastrointestinal tract (Lahri *et al.*, 1970; Bhat *et al.*, 1972, 1980; Applebaum *et al.*, 1980). It is noteworthy that in the studies of Bhat *et al.* (1972, 1980) and Applebaum *et al.* (1980), there was a mixed aerobic and anaerobic flora, whereas in the studies of Klipstein *et al.*, 1966a, 1973a), Gorbach *et al.* (1970), and Tomkins *et al.* (1975), although none of the papers give full bacteriological details, the predominant organisms appear to have been coliforms.

The mechanisms that determine the type and extent of the microbial flora in the small intestine are not well understood. Factors of importance are probably the bacterial load ingested, the nature of the diet (Klipstein and Corcino, 1974), the gastric pH, the secretory immune system of the gut, the flushing action of the flow of luminal contents, and the differing abilities of organisms to adhere to the mucosa. These factors have received very little attention. Cook (1978b) postulates that decrease in intestinal motility is the cause of the bacterial overgrowth in tropical sprue. This hypothesis, however, ignores the fact that although small intestinal transit time is prolonged in many cases of sprue, in others transit time is shortened (Baker, 1957; Paterson and Baker, 1958; Baker and Mathan, 1971;

Tomkins *et al.*, 1975), and in both situations bacterial overgrowth may be present. Moreover, it does not explain the bacterial overgrowth seen in many healthy control subjects.

The precise role of intestinal bacteria in the pathogenesis of tropical sprue is not clear. It should be noted that not all subjects with tropical sprue have abnormal numbers of bacteria in the jejunum (Nadel and Gardner, 1956; Lahri *et al.*, 1970; O'Brien and England, 1971; Bhat *et al.*, 1972, 1980), nor do all subjects with an abnormal flora have malabsorption (Lahri *et al.*, 1970; Gorbach *et al.*, 1970; Bhat *et al.*, 1972). However, the observed responses to antimicrobial therapy (see Section 4.5.5) suggest that bacterial overgrowth may play an important role in some cases.

To further elucidate this problem, Klipstein and his colleagues have undertaken extensive studies of toxin production by organisms isolated from patients with tropical sprue. They found that coliforms (*Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Escherichia coli*) isolated from the midjejunum of Puerto Rican patients with tropical sprue produced a toxin that caused fluid secretion when injected into rabbit ileal loop preparations (Klipstein *et al.*, 1973b). This toxin also produced histological changes in the rabbit loops, and it was suggested that it might be responsible for the histological changes seen in the jejunal mucosa of patients with tropical sprue (Klipstein and Schenk, 1975a). The toxin has been shown to be heat stable, to resist acid treatment, and to have a molecular weight in the region of 1000–10,000 (Klipstein and Engert, 1975). When the toxin was perfused in rat small intestine, net secretion of water and sodium resulted (Klipstein *et al.*, 1975). Similar toxigenic coliforms were isolated from four of five Haitian patients with sprue but not from those with tropical enteropathy (Klipstein *et al.*, 1976). In a further extension of this work, Klipstein *et al.* (1978) compared 12 patients with tropical sprue (nine from Puerto Rico and three from Haiti) with five patients with blind loop syndrome from Rochester, New York. Fourteen out of 16 strains of coliforms isolated from patients with sprue produced heat-stable and/or heat-labile toxins. These produced net water secretion in the rat model in the same concentration range as that at which the toxin of classical toxigenic strains of *Escherichia coli* (from patients with acute gastroenteritis) was active. On the other hand, coliform strains isolated from the patients with the blind loop syndrome, and those isolated from healthy individuals, only produced toxin effective in at least 1000-fold higher concentration. These authors suggest that the malabsorption and histological changes in tropical sprue may at least partly be accounted for by the presence of the toxin-producing organisms.

On the other hand, Albert (1978), also using the rabbit ileal loop technique, studied isolates from 12 south Indian patients with tropical sprue. In only three of the 12 patients were toxin-producing coliforms found in the jejunum (four out of 23 strains studied), and in only two out of seven patients sampled were toxin-

producing organisms found in the ileum (three out of 16 strains studied). It is clear that the bacterial flora in these patients are very different from those of the Caribbean patients described by Klipstein *et al.* (1973b). This lower prevalence of toxin-producing organisms may account for some of the differences seen in response to antibiotic treatment in the two areas (see Section 4.5.5). Further, if one cause of the sprue syndrome is overgrowth with toxin-producing organisms, then at least many of the south Indian cases would appear to be of a different etiology.

4.6.4e. Mycoplasma. Lev *et al.* (1969) studied jejunal aspirates and jejunal biopsies from patients with tropical sprue and obtained mycoplasma from six of 12 patients with sprue and two of 12 control subjects. Unfortunately, the organisms were not typed, and no follow-up work has been published by these investigators. In south India no mycoplasma could be recovered from jejunal secretions or jejunal biopsies of 23 patients with sprue or 28 controls, although 45% of all salivary specimens grew mycoplasma, indicating that the methodology was adequate (Bhat *et al.*, 1973). It must be concluded that there is presently no evidence to incriminate any mycoplasma as a cause of the sprue syndrome.

4.6.4f. Viruses. Manson-Bahr (1957) suggested that tropical sprue might be an infection with a virus "like the herpes virus, which can maintain itself unchanged for many years in the human body. . . ." A viral etiology could explain many of the peculiar features of tropical sprue including the long latent period, the occurrence of epidemics, the altered age-specific attack rate in second epidemic waves, the clustering of cases in space and time seen in epidemics, and the occurrence of fever at the onset of illness in a proportion of cases. Although most viral diseases are not limited by geographical boundaries, a vector-borne virus could well explain the peculiar geographical distribution of the disease (e.g., yellow fever). In this respect, it is of interest that in one village epidemic of sprue in south India, the attack rate correlated with the amount of time spent in the house and with the prevalence of bedbugs in the house (S. J. Baker and V. I. Mathan, unpublished observations, 1970), suggesting a possible vector.

A number of epidemics of gastroenteritis have been caused by viruses (Reimann, 1963; Estes and Graham, 1979). The two most studied viral agents are the 27-nm diameter Norwalk Agent and the 70-nm rotavirus (Kapikain *et al.*, 1972, 1978; Bishop *et al.*, 1973). However, these agents cause a short-lived illness from which recovery is usually complete with little evidence of persisting malabsorption. Attempts in Puerto Rico (Bayless *et al.*, 1966) and south India (Baker, 1971) to grow viruses from patients with tropical sprue have been unsuccessful.

In 1975, we found coronaviruslike particles in the stools of south Indian control subjects and patients with tropical sprue (Mathan *et al.*, 1975b). Because

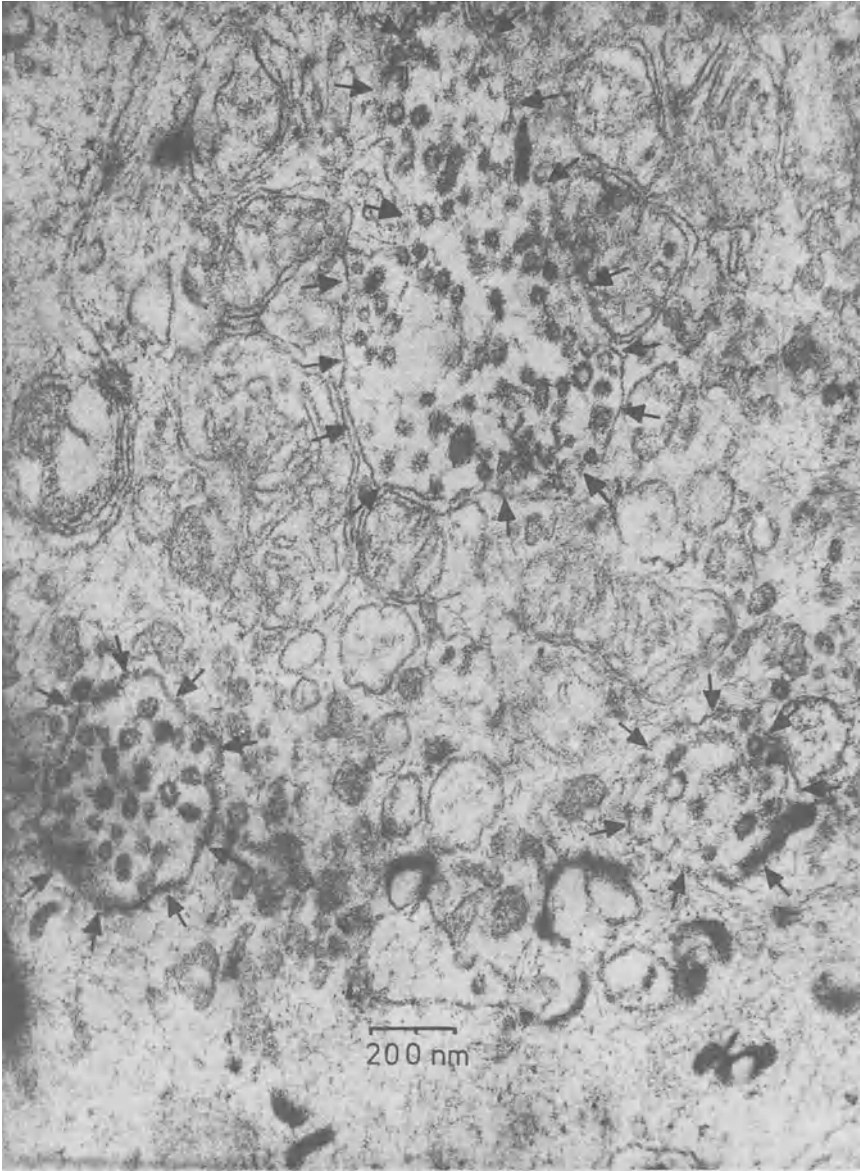


Figure 1. Electron micrograph of dying enterocyte from crypt area of an Indian patient with intestinal malabsorption and a gastroenterostomy, showing coronaviruslike vesicles (arrows).

of its occurrence in subjects without sprue, we tended to dismiss it as being of no significance. However, it is clear that in the case of many viruses, only a proportion of infected individuals develop clinical illness. It is therefore of interest that more recently we have observed vesicles containing coronaviruslike particles (Figure 1) in the pale-staining dying enterocytes of jejunal biopsies from a south Indian man with intestinal malabsorption who had also had a gastroenterostomy (Baker *et al.*, 1982). It was not possible to be sure how much of his malabsorption was caused by the mucosal disease and how much was caused by the gastroenterostomy. However, review of previously taken electron micrographs showed similar virus particles in pale-staining dying enterocytes in three of 12 patients with chronic tropical sprue, suggesting that this virus was either an opportunistic one, colonizing dying cells, or else, as seems more probable, it was causing premature enterocyte death. This virus must therefore be considered a serious candidate as one possible etiologic agent producing the intestinal damage that causes tropical sprue.

4.7. Summary and Conclusions

The rather confusing picture of the tropical sprue syndrome that emerges at the present time may perhaps be simplified by considering the pathogenesis in at least two steps, namely, the first step responsible for initiation of intestinal damage and the second which perpetuates or aggravates it.

The varying reports of response to treatment with folic acid, vitamin B₁₂, and antimicrobials are undoubtedly, in part, related to the total lack of controlled therapeutic trials. Nevertheless, when rapid improvement in intestinal function and structure occurs following institution of a given therapy, it is reasonable to assume that the administered therapy may have played some part in producing the improvement. On this basis, it appears that in certain patients with tropical sprue, folate and/or vitamin B₁₂ deficiency may be perpetuating or aggravating the intestinal damage, even though there is good reason to believe that they are not initiating factors. Protein deficiency may play a similar role. In a larger group of patients, the reported responses to antimicrobial therapy indicate that bacterial overgrowth, perhaps particularly, overgrowth with enterotoxigenic bacteria, may be a perpetuating or aggravating factor. Clearly, also, these factors may act alone or in combination with each other, so that treatment with one or any combination of folate, vitamin B₁₂, protein, and antimicrobials may be necessary to produce improvement.

The nature of the initiating agent(s) is less clear. Deficiency states can probably be eliminated for reasons given above. Studies from Klipstein's group suggest, but have not proven, that colonization with enterotoxigenic bacteria may also produce the initial damage (Klipstein, 1979). If this be so, since bacteria are ubiquitous, some other factor would have to be involved to explain

the unusual geographic distribution of the syndrome, such as a dietary peculiarity (Klipstein and Corcino, 1974). However, it is clear from studies in Vellore that many south Indian subjects with tropical sprue do not have toxin-producing organisms in their intestinal tract. In these cases, some other initiating agent(s) must be responsible. We believe, but have not yet proved, that at least one candidate agent is a coronaviruslike infection of the enterocytes. Thus, on the basis of available evidence, the hypothesis is advanced that there are at least two major forms of the syndrome of tropical sprue (Figure 2), viz:

1. Bacterial-initiated sprue (BIS), where colonization of the upper intestine with toxigenic bacteria plays a dominant role in initiation and, because of their continuing presence, the perpetuation of the disease.
2. Viral-initiated sprue (VIS), where the initiating agent is a coronaviruslike (or other viral) infection of the enterocytes which may, at times, also be complicated by secondary bacterial overgrowth.

Either form may be further complicated by folate or vitamin B₁₂ and perhaps protein deficiency.

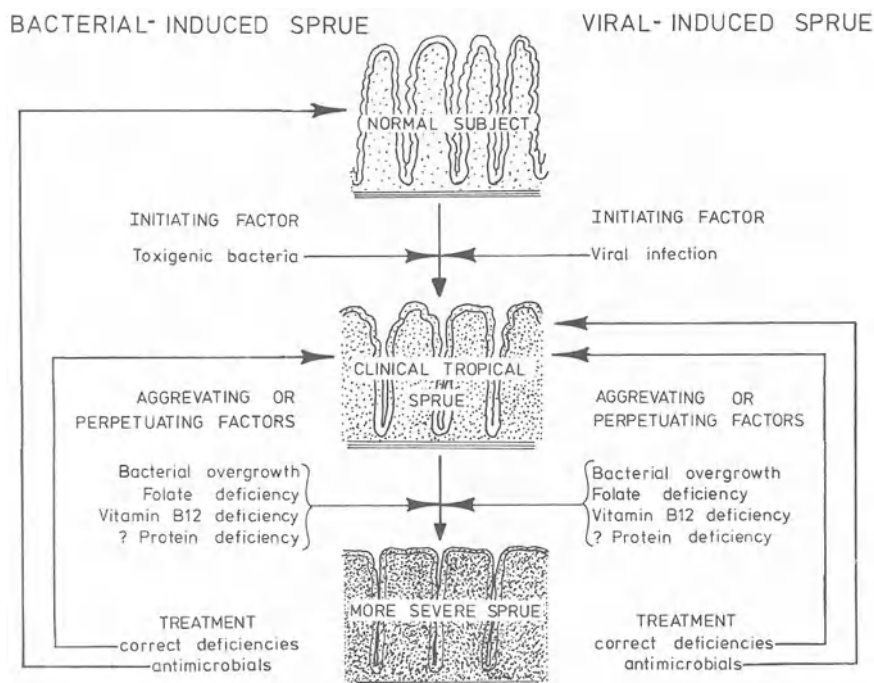


Figure 2. Diagrammatic representation of the suggested pathogenesis and response to treatment of two forms of tropical sprue—bacterial induced sprue (BIS) and viral induced sprue (VIS).

This hypothesis explains the variable responses to folate and vitamin B₁₂ supplementation [e.g., in cases where folate deficiency is exerting a major influence on intestinal function, folate therapy will have a marked effect (Spies *et al.*, 1946), but where it has only a minor effect or is absent, folate therapy will produce little or no change]. It also explains the apparent cure with antimicrobial therapy of many cases of tropical sprue (BIS) (e.g., in the Caribbean) and the failure of the same therapy in other cases (VIS) (e.g., in south India). Viral initiated sprue is probably responsible for the epidemics of sprue and the form of the disease with a long latent period. The few reports of "tropical sprue" in temperate zones may be cases of BIS syndrome where, for some reason, toxigenic coliforms have colonized the upper intestine.

Future research must be aimed at clarifying a number of points including the following:

1. The factors that control the microecology of the upper small intestine.
2. Whether BIS is indeed initiated by the bacteria themselves or whether some other initiating agent is involved and the bacterial overgrowth is only a secondary phenomenon.
3. The precise factors that determine the geographical distribution of BIS.
4. The fulfilling of Koch's postulates with regard to the role of the coronaviruslike agents, or other agents, in the initiation of VIS.
5. The mode(s) of transmission of VIS and its true geographical distribution.

When answers to these questions are available, we will understand much more about this baffling but fascinating syndrome and be in a better position to rationally treat and prevent it.

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REFERENCES

- Abbad y Lasierra, I., 1959, *Historia Geográfica, Civil y Natural de la Isle de San Juan de Puerto Rico*, University of Puerto Rico, San Juan.
- Abrams, G. D., Bauer, H., and Sprinz, H., 1963, Influence of the normal flora on mucosal morphology and cellular renewal in the ileum. A comparison of germ-free and conventional mice, *Lab. Invest.* 12:355-364.

- Albert, M. J., 1978, *Microflora of the Human Intestinal Tract in Health and Disease*, Ph.D. Thesis, University of Madras, Madras.
- Albert, M. J., Rajan, D., Bhat, P., Gammon, A., Mathan, V. I., and Baker, S. J., 1982, Intestinal luminal flora and vitamin B₁₂ malabsorption in tropical sprue, submitted for publication.
- Ament, M. E., Shimoda, S. S., Saunders, D. R., and Rubin, C. E., 1971, Pathogenesis of steatorrhea in three cases of small intestinal stasis syndrome, *Gastroenterology* 63:728–747.
- Angel, C. R., Guerra, R., Martinez, J., and Plough, I. C., 1963, Malabsorption of xylose in Puerto Rico, *Fed. Proc.* 22:550.
- Appelbaum, P. C., Moshal, M. G., Hift, W., and Chatterton, S. A., 1980, Intestinal bacteria in patients with tropical sprue, *S. Afr. Med. J.* 57:1081–1083.
- Ashcroft, M. T., 1970, Tropical sprue in the Caribbean, *Lancet* 2:724–725.
- Ashford, B. K., 1931, Relation of *Monilia psiloses* to tropical sprue and evaluation of fermentation of sugar as a criterion of specificity, *Puerto Rico J. Public Health Trop. Med.* 6:310–333.
- Ashford, B. K., 1932, *Tice's Practice of Medicine*, Volume 14, Prior, Hagerstown, Maryland, p. 173.
- Ayrey, F., 1948, Outbreaks of sprue during the Burma campaign, *Trans. R. Soc. Trop. Med. Hyg.* 41:377–406.
- Bahr, P. H., 1915, *A Report on Researches on Sprue in Ceylon, 1912–1914*, University Press, Cambridge.
- Baker, S. J., 1957, Idiopathic tropical steatorrhea: A report of 60 cases, *Indian J. Med. Sci.* 11:687–703.
- Baker, S. J., 1966, The recognition of vitamin B₁₂ and folate deficiency, *N.Z. Med. J. [Suppl.]* 65:884–892.
- Baker, S. J., 1967, Human vitamin B₁₂ deficiency, *World Rev. Nutr. Diet.* 8:62–126.
- Baker, S. J., 1971, Tropical sprue, *Br. Med. Bull.* 28:87–91.
- Baker, S. J., 1972, Vitamin B₁₂ and tropical sprue, *Br. J. Haematol.* 23 (Suppl.):135–146.
- Baker, S. J., 1973, Geographical variations in the morphology of small intestinal mucosa in apparently healthy individuals, *Pathol. Microbiol.* 39:222–237.
- Baker, S. J., 1976, Folate absorption, in: *Radioactive Tracer Techniques for the Study of Intestinal Absorption*, International Atomic Energy Agency, Vienna, pp. 192–206.
- Baker, S. J., 1977, Malnutrition and small intestinal disease, in: *Progress in Gastroenterology*, Volume III (G. B. J. Glass, ed.), Grune & Stratton, New York, pp. 563–592.
- Baker, S. J., and Mathan, V. I., 1968a, Natural history of tropical sprue in south India, in: *Tropical Medicine Conference (1967)* (J. H. Walters, ed.), Pitman, London, pp. 65–85.
- Baker, S. J., and Mathan, V. I., 1968b, Syndrome of tropical sprue in south India, *Am. J. Clin. Nutr.* 21:984–993.
- Baker, S. J., and Mathan, V. I., 1970, An epidemic of tropical sprue in southern India. II. Epidemiology, *Ann. Trop. Med. Parasitol.* 64:453–467.
- Baker, S. J., and Mathan, V. I., 1971, Tropical sprue in south India, in: *Tropical Sprue and Megaloblastic Anaemia*, Wellcome Trust Collaborative Study, Wellcome Trust, Churchill Livingstone, Edinburgh, London, pp. 189–260.
- Baker, S. J., and Mathan, V. I., 1972, Tropical enteropathy and tropical sprue, *Am. J. Clin. Nutr.* 25:1047–1055.
- Baker, S. J., and Rao, G. A., 1962, The use of isotopes in tropical sprue, in: *Radioisotopes in Tropical Medicine*, International Atomic Energy Agency, Vienna, pp. 45–66.
- Baker, S. J., Ignatius, M., Mathan, V. I., Vaish, S. K., and Chacko, C. C., 1962, Intestinal biopsy in tropical sprue, in: *Intestinal Biopsy*, Ciba Foundation Study Group No. 14, (G. E. W. Wolstenholme and M. P. Cameron, eds.), Churchill Livingstone, Edinburgh, pp. 84–118.
- Baker, S. J., Mathan, V. I., and Joseph, I., 1963, The epidemiology of tropical sprue, in: *Malab-*

- sorption Syndromes, Symposium Held at the Second World Congress Gastroenterology (Munich, 1962)*, (Schön, H., ed.), Karger, Basel, New York, pp. 4–7.
- Baker, S. J., Mathan, M., Mathan, V. I., Jesudoss, S., and Swaminathan, S. P., 1982, Chronic enterocyte infection with coronavirus, *Dig. Dis. Sci.*, submitted for publication.
- Balogopal, G., Jacob, R., Swarnabai, C., Kapadia, C. R., Mathan, V. I., and Baker, S. J., 1975, Secretion of exocrine pancreatic enzymes in patients with tropical sprue, following stimulation by a test meal, *Indian J. Med. Res.* 63:1138–1149.
- Ballingal, G., 1818, *Practical Observations on Fever, Dysentery and Liver Complaints as They Occurred Amongst European Troops in India*, Underwood, London.
- Banwell, J. G., and Gorbach, S. L., 1969, Tropical Sprue, *Gut* 10:328–333.
- Banwell, J. G., Hutt, M. S. R., Marsden, P. D., and Blackman, V., 1964, Malabsorption syndromes amongst African people in Uganda, *East Afr. Med. J.* 41:188–193.
- Banwell, J. G., Hutt, M. R. S., Leonard, P. J., Blackman, V., Connor, D. W., Marsden, P. D., and Campbell, J., 1967, Exocrine pancreatic disease and the malabsorption syndrome in tropical Africa, *Gut* 8:388–401.
- Banwell, J. G., Gorbach, S. L., Mitra, R., Cassells, J. S., Mazumder, D. N. G., Thomas, J., and Yardley, J. H., 1970, Tropical sprue and malnutrition in West Bengal. II. Fluid and electrolyte transport in the small intestine, *Am. J. Clin. Nutr.* 23:1559–1568.
- Bayless, T. M., 1964, Tropical sprue: A comparison with celiac disease, *Am. J. Dig. Dis.* 9:779–780.
- Bayless, T. M., Walter, W., and Barber, R., 1964, Disaccharidase deficiencies in tropical sprue, *Clin. Res.* 12:445.
- Bayless, T. M., Guardiola-Rotger, A., and Wheby, M. S., 1966, Tropical sprue: Viral cultures of rectal swabs, *Gastroenterology* 51:32–35.
- Bayless, T. M., Partin, J. S., and Partin, J. C., 1967, Serum precipitins to milk, gluten and rice in tropical sprue, *Johns Hopkins Med. J.* 120:310–316.
- Bayless, T. M., Wheby, M. S., and Swanson, V. L., 1968, Tropical sprue in Puerto Rico, *Am. J. Clin. Nutr.* 21:1030–1041.
- Bayless, T. M., Swanson, V. L., and Wheby, M. S., 1971, Jejunal histology and clinical status in tropical sprue and other chronic diarrheal disorders, *Am. J. Clin. Nutr.* 24:112–116.
- Begg, C., 1912, *Sprue, Its Diagnosis and Treatment*, Wright and Sons, Bristol.
- Beker, S., Valencia-Parparcen, J., Carbonnel, L., Bosch, V., de Bosch, N. F., and Moncada, P. R., 1961, Estudios funcionales e histopatologicos en el síndrome de malabsorción intestinal, *GEN* 15:461–476.
- Bernstein, L. H., Gutstein, S., Weiner, S., and Efron, G., 1970, The absorption and malabsorption of folic acid and its polyglutamates, *Am. J. Med.* 48:570–579.
- Bernstein, L. H., Lepow, H., Wagle, A., Doran, T., and Brand, B., 1973, Is tropical sprue an algal disease? *Gastroenterology* 64:697.
- Bevan, G., Engert, R., Klipstein, F. A., Maldonado, N., Rubulis, A., and Turner, M. D., 1974, Bile salt metabolism in tropical sprue, *Gut* 15:254–259.
- Bhat, P., Shantakumari, S., Rajan, D., Mathan, V. I., Kapadia, C. R., Swarnabai, C., and Baker, S. J., 1972, Bacterial flora of the gastrointestinal tract in south Indian control subjects and patients with tropical sprue, *Gastroenterology* 62:11–21.
- Bhat, P., Boulter, A. E., Rajan, D., Shantakumari, S., Kapadia, C. R., and Baker, S. J., 1973, Mycoplasma in the upper gastrointestinal tracts of southern Indian control subjects and patients with tropical sprue, *Am. J. Clin. Pathol.* 59:825–828.
- Bhat, P., Albert, M. J., Rajan, D., Ponniah, J., Mathan, V. I., and Baker, S. J., 1980, Bacterial flora of the jejunum: A comparison of luminal aspirate and mucosal biopsy, *J. Med. Microbiol.* 13:247–256.

- Bianchi, A., Chipman, D. W., Dreskin, A., and Rosenweig, N. S., 1970, Nutritional folic acid deficiency with megaloblastic changes in the small bowel epithelium, *N. Engl. J. Med.* 282:859–861.
- Bishop, R. F., Davidson, G. P., Holmes, I. H., and Ruck, B. J., 1973, Virus particles in epithelial cells of duodenal mucosa from children with acute gastroenteritis, *Lancet* 2:1281–1283.
- Booth, C. C., Stewart, J. S., Holmes, R., and Brackenbury, W., 1962, Dissecting microscope appearances of intestinal mucosa, in: *Intestinal Biopsy*, Ciba Foundation Study Group No. 14, (G. E. W. Wolstenholme and M. P. Cameron, eds.), Churchill, Livingstone, Edinburgh, pp. 2–19.
- Booth, C. C., Tabaqchali, S., and Mollin, D. L., 1968, Comparison of stagnant-loop syndrome with chronic tropical sprue, *Am. J. Clin. Nutr.* 21:1097–1109.
- Brandborg, L. L., Goldberg, S. B., and Breidenbach, W. C., 1970, Human coccidiosis—a possible cause of malabsorption, *N. Engl. J. Med.* 283:1306–1313.
- Brunser, O., Eidelman, S., and Klipstein, F. A., 1970, Intestinal morphology of rural Haitians. A comparison between overt tropical sprue and asymptomatic subjects, *Gastroenterology* 58:655–668.
- Burman, N. N., Sehgal, A. K., Chakravarti, R. N., Sodhi, J. S., and Chuttani, P. N., 1970, Morphological and absorption studies of small intestine in hookworm infestation (ankylostomiasis), *Indian J. Med. Res.* 58:317–325.
- Butterworth, C. E., Nadel, H., Perez-Santiago, E., Santini, R., and Gardner, F. H., 1957, Folic acid absorption, excretion and leucocyte concentration in tropical sprue, *J. Lab. Clin. Med.* 50:673–681.
- Butterworth, C. E., Baugh, C. M., and Krumdieck, C., 1969, A study of folate absorption and metabolism in man utilising carbon-14-labelled polyglutamates synthesized by the solid phase method, *J. Clin. Invest.* 48:1131–1142.
- Caldwell, W. L., Swanson, V. L., and Bayless, T. M., 1965, The importance and reliability of roentgenographic examination of the small bowel in patients with tropical sprue, *Radiology* 84:227–240.
- Cancio, M., and Menendes-Corrada, R., 1964, Absorption of medium chain triglycerides in tropical sprue, *Proc. Soc. Exp. Biol. Med.* 117:182–185.
- Caraka Samhita, 1949, *Volume III*, Shree Gulabkunverba Ayurvedic Society, Jamnagar, p. 1872.
- Carmel, R., Waxman, S., Epstein, B., Rausen, A., Jeffries, C., and Herbert, V., 1968, Acquired (?) selective malabsorption of B₁₂ in a family, in: *XII Congress International Society of Hematology*, New York, Grune & Stratton, New York, p. 90.
- Cassells, J. S., Banwell, J. G., Gorbach, S. L., Mitra, R., and Mazumder, D. N. G., 1970, Tropical sprue and malnutrition in West Bengal. IV. Bile salt deconjugation in tropical sprue, *Am. J. Clin. Nutr.* 23:1579–1581.
- Chacko, C. J. G., Job, C. K., Johnson, S., and Baker, S. J., 1961, Histopathological changes in the upper jejunum in tropical malabsorption syndrome studied by transoral biopsy, *Indian J. Pathol. Bacteriol.* 4:203–213.
- Chacko, C. J. G., Paulson, K. A., Mathan, V. I., and Baker, S. J., 1969, The villus architecture of the small intestine in the tropics: A necropsy study, *J. Pathol.* 98:146–151.
- Chanarin, I., Anderson, B. B., and Mollin, D. L., 1958, The absorption of folic acid, *Br. J. Haematol.* 4:156–166.
- Chauduri, R. N., and Rai Chauduri, M. N., 1944, Parasprue, *Indian Med. Gaz.* 79:404–408.
- Chuttani, H. K., Puri, S. H., and Misra, R. C., 1967, Small intestine in hookworm disease, *Gastroenterology* 53:381–388.
- Chuttani, H. K., Kasthuri, D., and Misra, R. C., 1968a, Course and prognosis of tropical sprue, *J. Trop. Med. Hyg.* 71:96–99.

- Chuttani, H. K., Mehra, M. L., and Misra, R. C., 1968b, Small bowel in hypoproteinemic states, *Scand. J. Gastroenterol.* 3:529–536.
- Colwell, E. J., Welsh, J. D., Legters, L. J., and Proctor, R. F., 1968, Jejunal morphological characteristics in South Vietnamese residents. A preliminary report, *J.A.M.A.* 206:2273–2276.
- Cook, G. C., 1972, Impairment of D-xylose absorption in Zambian patients with systemic bacterial infections, *Am. J. Clin. Nutr.* 25:490–493.
- Cook, G. C., 1974a, Malabsorption in Africa, *Trans. R. Soc. Trop. Med. Hyg.* 68:419–435.
- Cook, G. C., 1974b, Jejunal absorption rates of glucose, glycine, and glycylglycine in Zambian African adults with malnutrition, *Br. J. Nutr.* 32:503–513.
- Cook, G. C., 1978a, Tropical sprue: Implications of Manson's concept, *J. R. Coll. Physicians Lond.* 12:329–349.
- Cook, G. C., 1978b, Delayed small-intestinal transit in tropical malabsorption, *Br. Med. J.* 2:238–240.
- Cook, G. C., Kajubi, S. K., and Lee, F. D., 1969, Jejunal morphology of the African in Uganda, *J. Pathol.* 98:157–169.
- Cook, G. C., Asp, N-G., and Dahlqvist, A., 1973, Activities of brush border lactase, acid-galactosidase, and heterogalactosidase in the jejunum of the Zambian African, *Gastroenterology* 64:405–410.
- Cooke, W. T., Fone, D. J., Cox, E. V., Meynell, M. J., and Gaddie, R., 1963, Adult folic acid deficiency of unknown aetiology: Temperate sprue, *Gut* 4:292–298.
- Corcino, J. J., 1975, Recent advances in tropical sprue, in: *Intestinal Absorption and Malabsorption* (T. Z. Csaky, ed.), Raven Press, New York, pp. 285–299.
- Corcino, J. J., Maldonado, M., and Klipstein, F. A., 1973, Intestinal perfusion studies in tropical sprue. I. Transport of water, electrolytes, and D-xylose, *Gastroenterology* 65:192–198.
- Corcino, J. J., Coll, G., and Klipstein, F. A., 1975, Pteroylglutamic acid malabsorption in tropical sprue, *Blood* 45:577–580.
- Corcino, J. J., Reisenauer, A. M., and Halsted, C. H., 1976, Jejunal perfusion of simple and conjugated folates in tropical sprue, *J. Clin. Invest.* 58:298–305.
- Cowan, B., 1972, Malnutrition and malabsorption: Studies in the Punjab, *Am. J. Clin. Nutr.* 25:1234–1235.
- Cowan, B., Satija, V. K., and Malvea, B. P., 1971, Steatorrhea in the Punjab: The results of a village survey, *J. Trop. Med. Hyg.* 74:137–140.
- Dean, A. F., and Jones, T. C., 1972, Seasonal gastroenteritis and malabsorption at an American military base in the Philippines I. Clinical and epidemiologic investigations of the acute illness, *Am. J. Epidemiol.* 95:111–127.
- Deo, M. G., and Ramalingaswami, V., 1965, Reaction of the small intestine to induced protein malnutrition in rhesus monkeys—a study of cell population kinetics of the jejunum, *Gastroenterology* 49:150–157.
- Desai, H. G., Parekh, D. V., and Jeejeebhoy, K. N., 1966, Bacteriological study of small intestinal fluid in tropical sprue, *J. Assoc. Physicians India* 14:203–205.
- Desai, H. G., Borkar, A. V., Pathare, S. M., Dighe, P. K., and Jeejeebhoy, K. N., 1969, "Flat" jejunal mucosa in the tropics, *Indian J. Med. Sci.* 23:1–5.
- Desai, H. G., Zaveri, M. P., and Antia, F. P., 1972, Bile salt metabolism in tropical sprue, *Indian J. Med. Res.* 60:1170–1174.
- Donaldson, R. M., 1962, Malabsorption of Co⁶⁰ labelled cyanocobalamin in rats with intestinal diverticula. I. Evaluation of possible mechanisms, *Gastroenterology* 43:271–281.
- Donaldson, R. M., 1965, Studies on the pathogenesis of steatorrhea in the blind loop syndrome, *J. Clin. Invest.* 44:1815–1825.
- Duque, E., Bolanos, O., Lotero, H., and Mayoral, G., 1975, Enteropathy in adult protein malnutrition—light microscopic findings, *Am. J. Clin. Nutr.* 28:901–913.

- Duran, C., 1924, Some observations of "sprue" in Costa Rica, *Am. J. Trop. Med.* 4:393-395.
- Einstein, L. P., MacKay, D. M., and Rosenberg, I. H., 1972, Pediatric xylose malabsorption in East Pakistan: Correlation with age, growth retardation and weanling diarrhea, *Am. J. Clin. Nutr.* 25:1230-1233.
- Elder, H. H. A., 1947, Clinical features, diagnosis and treatment of sprue, *Trop. Med. Hyg.* 50:212-218.
- England, N. W. J., 1968, Intestinal pathology of tropical sprue, *Am. J. Clin. Nutr.* 21:962-975.
- England, N. W. J., and O'Brien, W., 1966, Appearances of the jejunal mucosa in acute tropical sprue in Singapore, *Gut* 7:128-139.
- Estes, M. K., and Graham, D. Y., 1979, Epidemic viral gastroenteritis, *Am. J. Med.* 66:1001-1007.
- Fairley, N. H., 1930, Sprue, its applied pathology, biochemistry and treatment, *Trans. R. Soc. Trop. Med. Hyg.* 24:131-186.
- Fairley, N. H., 1934, Tropical sprue and its modern treatment, *Br. Med. J.* 3:1192-1194.
- Fairley, N. H., 1936, Tropical sprue with special reference to intestinal absorption, *Trans. R. Soc. Trop. Med. Hyg.* 30:9-32.
- Falaiye, J. M., 1969, Survey of *D*-xylose absorption and nutritional deficiencies in Nigeria, *West Afr. Med. J.* 18:71-74.
- Falaiye, J. M., 1970, Tropical sprue in Nigeria, *J. Trop. Med. Hyg.* 73:119-125.
- Fayrer, J., 1881, *Tropical Dysentery and Chronic Diarrhea*, Churchill, Livingstone, Edinburgh, pp. 127-171.
- Floch, M. H., Thomasset, R. W., Cox, R. S., and Sheehy, T. W., 1963, The gastric mucosa in tropical sprue, *Gastroenterology* 44:567-577.
- Forshaw, J., Moorehouse, E. H., and Harwood, L., 1964, Megaloblastic anemia due to dietary deficiency, *Lancet* 1:1004-1008.
- Foy, H., and Kondi, A., 1971, Megaloblastic anemia in East Africa, in: *Tropical Sprue and Megaloblastic Anaemia*, Wellcome Trust Collaborative Study, Wellcome Trust, Churchill Livingstone, Edinburgh, London, pp. 261-268.
- French, J. M., 1955, Aetiology and mechanism of steatorrhea, *Postgrad. Med. J.* 31:299-309.
- French, J. M., Gaddie, R., and Smith, N. M., 1956, Tropical sprue, a study of seven cases and their response to combined chemotherapy, *Q. J. Med.* 25:333-351.
- Galloway, D. J., 1905a, Discussions on sprue and hill diarrhoea, *Br. Med. J.* 2:1284.
- Galloway, D. J., 1905b, Some clinical notes on the aetiology of sprue, *J. Trop. Med. Hyg.* 8:289-293.
- Garbutt, J. T., Heaton, K. W., Lack, L., and Tyor, M., 1969, Increased ratio of glycine to taurine conjugated bile salts in patients with ileal disorders, *Gastroenterology* 56:711-720.
- Garcia, S., 1968, Malabsorption and malnutrition in Mexico, *Am. J. Clin. Nutr.* 21:1066-1076.
- Gardner, F. H., 1956, A malabsorption syndrome in military personnel in Puerto Rico, *Arch. Intern. Med.* 98:44-60.
- Gardner, F. H., 1958, Tropical sprue, *N. Engl. J. Med.* 258:791-796, 835-842.
- Gerson, C. D., Kent, T. H., and Saha, J. R., 1971a, Recovery of small intestinal structure and function after residence in the tropics. II. Studies in Indians and Pakistanis living in New York City, *Ann. Intern. Med.* 75:41-48.
- Gerson, C. D., Cohen, N., Hepner, G. W., Brown, N., Herbert, V., and Janowitz, H. D., 1971b, Folic acid absorption in man: Enhancing effect of glucose, *Gastroenterology* 61:224-227.
- Ghitis, J., Tripathy, K., and Mayoral, G., 1967, Malabsorption in the tropics. 2. Tropical sprue versus primary protein malnutrition: Vitamin B₁₂ and folic acid studies, *Am. J. Clin. Nutr.* 20:1206-1211.
- Goldberger, J., Wheeler, G. A., and Sydenstricker, E., 1920, Pellagra incidence in relation to sex, age, season, occupation and "disabling sickness" in seven cotton mill villages of South Carolina during 1916, *Public Health Rep.* 35:1650-1664.

- Goldstein, F., Dammin, G. J., Mandle, R. J., and Wirts, C. W., 1972, Clinical syndrome resembling tropical sprue in lifelong residents of temperate zone, *Am. J. Dig. Dis.* 17:407–414.
- Gorbach, S. L., Banwell, J. G., Mitra, R., Chatterjee, B. D., Jacobs, B., and Mazumder, D. N. G., 1969, Bacterial contamination of the upper small bowel in tropical sprue, *Lancet* 1:74–77.
- Gorbach, S. L., Banwell, J. G., Jacobs, B., Chatterjee, B. D., Mitra, R., Sen, N. N., and Mazumder, D. N. G., 1970, Tropical sprue and malnutrition in West Bengal. 1. Intestinal microflora and absorption, *Am. J. Clin. Nutr.* 23:1545–1558.
- Gough, K. R., Read, A. E., McCarthy, C. F., and Waters, A. H., 1963, Megaloblastic anaemia due to nutritional deficiency of folic acid, *Q. J. Med.* 32:243–256.
- Grant, A., 1854, Remarks on hill diarrhoea and dysentery, *Indian Ann. Med. Sci.* 1:311–334.
- Gray, G. M., Walter, W. M., and Colver, E. H., 1968, Persistent deficiency of intestinal lactase in apparently cured tropical sprue, *Gastroenterology* 54:552–558.
- Guerra, R., Wheby, M. S., and Bayless, T. M., 1965, Long term antibiotic therapy in tropical sprue, *Ann. Intern. Med.* 63:619–634.
- Haghighi, P., and Nasr, K., 1975, Tropical sprue: Subclinical and idiopathic enteropathy, *Pathol. Annu.* 10:177–203.
- Halsted, C. H., Sheirs, S., Sourial, N., and Patwardhan, V. N., 1969, Small intestinal structure and absorption in Egypt. Influence of parasitism and pellagra, *Am. J. Clin. Nutr.* 22:744–754.
- Halsted, C. H., Robles, E. A., and Mezey, E., 1973, Intestinal malabsorption in folate deficient alcoholics, *Gastroenterology* 64:526–632.
- Harper, G. P., 1972, Xylose malabsorption and growth retardation in East Pakistani children, *Am. J. Clin. Nutr.* 25:1227–1229.
- Hartman, R. S., Butterworth, C. E., Hartman, R. E., Crosby, W. H., and Shirai, A., 1960, An electron microscopic investigation of the jejunal epithelium in sprue, *Gastroenterology* 38:506–516.
- Heiner, D. C., Lahey, M. E., and Wilson, J. F., 1964, Precipitins to wheat gliadin in the study of celiac disease, *Am. J. Dig. Dis.* 9:786–791.
- Hellier, M. D., Radhakrishnan, A. N., Ganapathy, V., Gammon, A., and Baker, S. J., 1976, Intestinal absorption in normal Indian and English people, *Br. Med. J.* 1:186–188.
- Herbert, V., 1962, Experimental nutritional folate deficiency in man, *Trans. Assoc. Am. Physicians* 75:307–320.
- Hermos, J. A., Adams, W. H., Liu, Y. K., Sullivan, L. W., and Trier, J. S., 1972, Mucosa of the small intestine in folate deficient alcoholics, *Ann. Intern. Med.* 76:957–965.
- Hillary, W., 1759, *Observations on the Changes of the Air and Concomitant Epidemical Diseases in the Island of Barbados*, Hitch and Hawes, London, p. 277.
- Hoffbrand, A. V., Necheles, T. F., Maldonado, N., Horta, E., and Santini, R., 1969, Malabsorption of folate polyglutamates in tropical sprue, *Br. Med. J.* 2:543–547.
- Horta, E. O., Maldonado, N., Fradera, J., Santini, R., and Vélez-García, E., 1971, Response to tropical sprue to poorly absorbed sulfonamides and oxytetracycline, *Am. J. Clin. Nutr.* 23:1327–1336.
- Jeejeebhoy, K. N., Desai, H. G., Noronha, J. M., Antia, F. P., Parekh, D. V., 1966, Idiopathic tropical diarrhea with or without steatorrhea (tropical malabsorption syndrome), *Gastroenterology* 51:333–344.
- Jeejeebhoy, K. N., Ramanath, P., Mehan, K. P., Pathare, S. M., Parekh, D. V., Nadkarni, G. D., and Ganatra, R. D., 1967, A simple method of estimating folic acid absorption (A modified faecal excretion method), *J. Nucl. Med.* 8:40–49.
- Jeejeebhoy, K. N., Desai, H. G., Borkar, A. V., Deshpande, V., and Pathare, S. M., 1968, Tropical malabsorption syndrome in West India, *Am. J. Clin. Nutr.* 21:994–1006.
- Jeejeebhoy, K. N., Samuel, A. M., Singh, B., Nadkarne, G. D., Desai, H. G., Borkari, A. V., and

- Mani, L. D., 1969, Metabolism of albumin and fibrinogen in patients with tropical sprue, *Gastroenterology* 56:252–267.
- Jensen, H., Jarnum, S., and Hart Hansen, J. P., 1966, Gastrointestinal protein loss and intestinal function in the nephrotic syndrome, *Nephron* 3:209–220.
- Jones, C. T., Dean, A. G., and Parker, G. W., 1972, Seasonal gastroenteritis and malabsorption at an American military base in the Philippines. II. Malabsorption following the acute illness, *Am. J. Epidemiol.* 95:128–139.
- Juttijudata, P., Keusch, G. T., Troncale, F. J., Plaut, A. G., Buchanan, R. D., and Bhamarapravathi, N., 1969, Tropical sprue in Thailand. Report of the first documented case, *Am. J. Trop. Med.* 18:618–620.
- Kapadia, C. R., and Baker, S. J., 1973, The increased rate of loss, and non-exponential excretion of bile salts in patients with tropical sprue and ileectomy, *Aust. N.Z. J. Med.* 3:260–263.
- Kapadia, C. R., Radhakrishnan, A. N., Mathan, V. I., and Baker, S. J., 1971a, Studies on bile salt deconjugation in patients with tropical sprue, *Scand. J. Gastroenterol.* 6:29–32.
- Kapadia, C. R., Radhakrishnan, A. N., Mathan, V. I., and Baker, S. J., 1971b, A study of the ratios of bile salt conjugates of glycine to taurine in the jejunum and ileum in patients with tropical sprue, *Scand. J. Gastroenterol.* 6:357–361.
- Kapadia, C. R., Bhat, P., Jacob, E., and Baker, S. J., 1975, Viatamin B₁₂ absorption—a study of intraluminal events in control subjects and patients with tropical sprue, *Gut* 16:988–993.
- Kapikain, A. Z., Wyatt, R. G., Dolin, R., Thornhill, T. S., Kalica, A. R., and Chanock, R. M., 1972, Visualization by immune electron microscopy of a 27nm particle associated with acute infections non-bacterial gastroenteritis, *J. Virol.* 10:1075–1081.
- Kapikain, A. Z., Yolken, R. H., Wyatt, R. G., Kalica, A. R., Chanock, R. M., and Kim, H. W., 1978, Viral diarrhea. Etiology and control, *Am. J. Clin. Nutr.* 31:2219–2236.
- Keele, K. D., and Bound, J. P., 1946, Sprue in India, *Br. Med. J.* 1:77:81.
- Kendall, M. J., and Bayley, T. J., 1971, Temperate sprue, *Postgrad. Med. J.* 47:680–683.
- Kent, T. H., and Lindenbaum, J., 1967, Correlation of jejunal function and morphology in patients with acute and chronic diarrhea in East Pakistan, *Gastroenterology* 52:966–971.
- Ketelaer, V., 1669, *Commentarius Medicus de Aphis Nostratibus Seu Belgarum Sprouw*, reprinted 1715, Bernard, Amsterdam.
- Keusch, G. T., Plaut, A. G., and Troncale, F. J., 1970, The interpretation and significance of the xylose tolerance test in the tropics, *J. Lab. Clin. Med.* 75:558–565.
- Klipstein, F. A., 1964a, Antibiotic therapy in tropical sprue, the role of dietary folic acid in hematological remission associated with oral antibiotic therapy, *Ann. Intern. Med.* 61:721–728.
- Klipstein, F. A., 1964b, Tropical sprue in New York City, *Gastroenterology* 47:457–470.
- Klipstein, F. A., 1967a, Intestinal folate conjugase activity in tropical sprue, *Am. J. Clin. Nutr.* 20:1004–1009.
- Klipstein, F. A., 1967b, Tropical sprue—an iceberg disease, *Ann. Intern. Med.* 66:622–623.
- Klipstein, F. A., 1968, Progress in gastroenterology: Tropical sprue, *Gastroenterology* 54:275–293.
- Klipstein, F. A., 1969, Absorption of physiologic doses of folic acid in subjects with tropical sprue responding to tetracycline therapy, *Blood* 34:191–202.
- Klipstein, F. A., 1970, Recent advances in tropical malabsorption, *Scand. J. Gastroenterol.* 5(Suppl. 6):93–114.
- Klipstein, F. A., 1971, Tropical sprue in the Western Hemisphere, in: *Tropical Sprue and Megaloblastic Anaemia*, Wellcome Trust Collaborative Study, Wellcome Trust, Churchill Livingstone, Edinburgh, London, pp. 129–158.
- Klipstein, F. A., 1972, Folate in tropical sprue, *Br. J. Haematol.* 23(Suppl.):119–133.
- Klipstein, F. A., 1979, Sprue and subclinical malabsorption in the tropics, *Lancet* 1:277–278.
- Klipstein, F. A., and Baker, S. J., 1970, Regarding the definition of tropical sprue, *Gastroenterology* 58:717–721.

- Klipstein, F. A., and Corcino, J. J., 1974, Seasonal occurrence of overt and subclinical tropical malabsorption in Puerto Rico, *J. Am. Trop. Med. Hyg.* 23:1189–1196.
- Klipstein, F. A., and Corcino, J. J., 1977, Factors responsible for weight loss in tropical sprue, *Am. J. Clin. Nutr.* 30:1703–1708.
- Klipstein, F. A., and Engert, R. F., 1975, Enterotoxigenic intestinal bacteria in tropical sprue. III. Preliminary characterization of *Klebsiella pneumoniae* enterotoxin, *J. Infect. Dis.* 132:200–203.
- Klipstein, F. A., and Falaiye, J. M., 1969, Tropical sprue in expatriates from the tropics living in the continental United States, *Medicine* 48:475–491.
- Klipstein, F. A., and Samloff, I. M., 1966, Folate synthesis by intestinal bacteria, *Am. J. Clin. Nutr.* 19:237–246.
- Klipstein, F. A., and Schenk, E. A., 1975a, Enterotoxigenic intestinal bacteria in tropical sprue. II. Effect of the bacteria and their enterotoxins on intestinal structure, *Gastroenterology* 68(1):642–655.
- Klipstein, F. A., and Schenk, E. A., 1975b, Prototheca and sprue, *Gastroenterology* 69:1372–1373.
- Klipstein, F. A., Samloff, I. M., and Schenk, E. A., 1966a, Tropical sprue in Haiti, *Ann. Intern. Med.* 64:575–594.
- Klipstein, F. A., Schenk, E. A., and Samloff, I. M., 1966b, Folate repletion associated with oral tetracycline therapy in tropical sprue, *Gastroenterology* 51:317–332.
- Klipstein, F. A., Samloff, I. M., Smarth, G., and Schenk, E. A., 1968, Malabsorption and malnutrition in rural Haiti, *Am. J. Clin. Nutr.* 21:1042–1052.
- Klipstein, F. A., Samloff, I. M., Smarth, G., and Schenk, E. A., 1969, Treatment of overt and subclinical malabsorption in Haiti, *Gut* 10:315–322.
- Klipstein, F. A., Beauchamp, I., Corcino, J. J., Maldonado, M., Rubio, C., and Schenk, E. A., 1972, Nutritional status and intestinal function among rural populations of the West Indies. II. Barrio Nuevo, Puerto Rico, *Gastroenterology* 63:758–767.
- Klipstein, F. A., Rubio, C., Montas, F., Tomasini, J., and Castillo, R. G., 1973a, Nutritional status and intestinal function among populations of the West Indies. III. Barrio Cabreto, Dominican Republic, *Am. J. Clin. Nutr.* 26:87–95.
- Klipstein, F. A., Holdeman, L. V., Corcino, J. J., Moore, W. E. C., 1973b, Enterotoxigenic intestinal bacteria in tropical sprue, *Ann. Intern. Med.* 79:632–641.
- Klipstein, F. A., Horowitz, I. R., Engert, R. F., Schenk, E. A., 1975, Effect of *Klebsiella pneumoniae* enterotoxin on intestinal transport in the rat, *J. Clin. Invest.* 56:799–807.
- Klipstein, F. A., Short, H. B., Engert, R. F., Weaver, J. L., 1976, Contamination of the small intestine by enterotoxigenic coliform bacteria among the rural population of Haiti, *Gastroenterology* 70:1035–1041.
- Klipstein, F. A., Engert, R. F., and Short, H. B., 1978, Enterotoxigenicity of colonising coliform bacteria in tropical sprue and blind-loop syndrome, *Lancet* 2:342–344.
- Lahri, K., Bhujwala, R. A., Sama, S. K., and Tandon, B. N., 1970, Bacteriological study of jejunal fluid in idiopathic tropical malabsorption syndrome, *Indian J. Med. Res.* 58:1428–1432.
- Layrisse, M., Blumenfeld, N., Carbonnell, L., Desenne, J., and Roche, M., 1964, Intestinal absorption tests and biopsy of the jejunum in subjects with heavy hookworm infestation, *Am. J. Trop. Med. Hyg.* 13:297–305.
- Lev., M., Alexander, R., Levenson, S., Hepner, G., Gerson, C., Corcino, J., Janowitz, H. D., and Herbert, V., 1969, Mycoplasma in the small intestine in tropical sprue, *Clin. Res.* 17:595.
- Lindenbaum, J., 1965, Malabsorption during and after recovery from acute intestinal infection, *Br. Med. J.* 2:326–329.
- Lindenbaum, J., 1968, Small intestine dysfunction in Pakistanis and Americans resident in Pakistan, *Am. J. Clin. Nutr.* 21:1023–1029.

- Lindenbaum, J., 1973, Role of malabsorption in nutrition in: *Disaster in Bangladesh* (L. C. Chen, ed.), Oxford University Press, London, pp. 52–66.
- Lindenbaum, J., Jamiul Alam, A. K. M., and Kent, T. H., 1966a, Subclinical small intestinal disease in East Pakistan, *Br. Med. J.* 11:1616–1619.
- Lindenbaum, J., Kent, T. H., and Sprinz, H., 1966b, Malabsorption and jejunitis in American Peace Corps volunteers in Pakistan, *Ann. Intern. Med.* 65:1201–1209.
- Lindenbaum, J., Gerson, C. D., and Kent, T. H., 1971, Recovery of small intestinal structure and function after residence in the tropics. 1. Studies in Peace Corps volunteers, *Ann. Intern. Med.* 74:218–222.
- Lindenbaum, J., Harmon, J. W., and Gerson, C. D., 1972, Subclinical malabsorption in developing countries, *Am. J. Clin. Nutr.* 25:1056–1061.
- Lopez, G. C., Milanes, F., Spies, T. C., Toca, R. L., Aramburu, T., and Lopez, H., 1949, Association of hypoproteinemia with severe tropical sprue, *Am. J. Med. Sci.* 218:660–668.
- MacLean, W. C., 1886, *Diseases of Tropical Climates*, MacMillan, London, pp. 157–173.
- Maldonado, N., Horta, E., Guerra, R., and Pérez-Santiago, E., 1969, Poorly absorbed sulfonamides in the treatment of tropical sprue, *Gastroenterology* 57:559–568.
- Manson, P., 1880, Notes on sprue, in: *China, Imperial Maritime Customs, Special Series No. 2, Medical Reports for the Half Year Ended 31st March, 1880*, (19th issue), Statistical Department of the Inspectorate General, Shanghai, pp. 33–37.
- Manson-Bahr, P., 1957, Reflections on the etiology of tropical sprue (the present position), *Z. Tropenmed. Parasitol.* 8:174–181.
- Marin, G. A., Clark, M. L., and Senior, J. R., 1969, Studies of malabsorption occurring in patients with Laennec's cirrhosis, *Gastroenterology* 65:727–736.
- Mata, L. J., Jimenez, F., Cordon, M., Rosales, R., Prera, E., Schneider, R., and Viteri, F., 1972a, Gastrointestinal flora of children with protein-calorie malnutrition, *Am. J. Clin. Nutr.* 25:1118–1126.
- Mata L. J., Urrutia, J. J., Albertazzi, C., Pellecer, O., and Arellano, E., 1972b, Influence of recurrent infections on nutrition and growth of children in Guatemala, *Am. J. Clin. Nutr.* 25:1267–1275.
- Mathan, M., Mathan, V. I., and Baker, S. J., 1975a, An electron microscopic study of jejunal mucosal morphology in control subjects and in patients with tropical sprue in southern India, *Gastroenterology* 68:17–32.
- Mathan, M., Mathan, V. I., Swaminathan, S. P., Yesudoss, S., and Baker, S. J., 1975b, Pleomorphic virus-like particles in human feces, *Lancet* 1:1068–1069.
- Mathan, V. I., and Baker, S. J., 1968, Epidemic tropical sprue and other epidemics of diarrhea in south Indian villages, *Am. J. Clin. Nutr.* 21:1077–1087.
- Mathan, V. I., and Baker, S. J., 1970, An epidemic of tropical sprue in southern India. I. Clinical features, *Ann. Trop. Med. Parasitol.* 64:439–451.
- Mathan, V. I., and Baker, S. J., 1971, The epidemiology of tropical sprue, in: *Tropical Sprue and Megaloblastic Anaemia*, Wellcome Trust Collaborative Study, Churchill Livingstone, Edinburgh, London, pp. 159–188.
- Mathan, V. I., Ignatius, M., and Baker, S. J., 1966, A household epidemic of tropical sprue, *Gut* 7:490–496.
- Mathan, V. I., Joseph, S., and Baker, S. J., 1969, Tropical sprue in children. A syndrome of idiopathic malabsorption, *Gastroenterology* 56:556–570.
- Mayoral, L. G., Tripathy, K., Garcia, F. T., Klahr, S., Bolanos, D., and Ghitis, J., 1967, Malabsorption in the tropics: A second look. 1. The role of protein malnutrition, *Am. J. Clin. Nutr.* 20:866–883.
- Mayoral, L. G., Tripathy, K., Garcia, F. T., Bolanos, O., and Ghitis, J. 1968, Protein malnutrition-induced malabsorption and other enteropathies in Colombia, *Am. J. Clin. Nutr.* 21:1053–1065.

- Mayoral, L. G., Tripathy, K., Bolanos, O., Lotero, H., Duque, E., Garcia, F. T., and Ghitis, J., 1972, Intestinal, functional and morphologic abnormalities in severely protein-malnourished adults, *Am. J. Clin. Nutr.* 25:1084–1091.
- McLeod, G. M., and Wiggins, H. S., 1968, Bile salts in small intestinal contents after ileal resection and in other metabolic syndromes, *Lancet* 1:873–876.
- Mehta, S., Wadhwa, U. N., Prakash, A., and Chuttani, P. N., 1968, Small bowel function in severe chronic diarrhea in children, *J. Assoc. Physicians India* 16:343–346.
- Mehta, S. K., Bhargava, S. S., Khurana, K. K., Mysorekar, N. R., and Chuttani, P. N., 1971, Influence of dietary intake of fat on faecal fat excretion in tropical sprue, *Indian J. Med. Res.* 59:1435–1441.
- Mehta, S. K., Chakravarti, R. N., Nain, C. K., Das, S. K., Bhagwat, A. G., and Mehta, S., 1979, Primate model of sprue-like syndrome, *Israel J. Med. Sci.* 15:348–355.
- Meisel, J. L., Perera, D. R., Meligro, C., and Rubin, C. E., 1976, Overwhelming watery diarrhea, associated with cryptosporidium in an immunosuppressed patient, *Gastroenterology* 70:1156–1160.
- Menéndez-Corrada, R., and Belaval, M. E., 1968, Gluten antibodies and tropical sprue, *Am. J. Dig. Dis.* 13:987–993.
- Meyer, L. M., Suarez, R. M., Buso, R., Sabater, J., and Suarez, R. M., 1953, Oral administration of Co⁶⁰ vitamin B₁₂ in tropical sprue and hepatic cirrhosis and diarrhea, *Proc. Soc. Exp. Biol. Med.* 83:681–683.
- Mickelson, M. J., and Klipstein, F. A., 1975, Enterotoxigenic intestinal bacteria in tropical sprue. IV. Effect of linoleic acid on growth interrelationships of *Lactobacillus acidophilus* and *Klebsiella pneumoniae*, *Infect. Immun.* 12:1121–1126.
- Milanes, F., 1960, Changes in the epidemiology and clinical features of sprue in Cuba between 1927 and 1957, *Arch. Hosp. Univ. Habana* 12:125–136.
- Milanes, F., Curbelo, A., Rodriguez, A., Kouri, P., and Spies, T. D., 1946, A note on Bacteriological and parasitic studies of the intestinal contents of patients with sprue, *Gastroenterology* 7:306–313.
- Miller, M. D., Loftus, P. M., Lohr, D. C., Reynolds, R. D., Bratton, J. L., Hanson, J. P., and Keil, P. G., 1974, Tropical sprue in South Vietnam, *Milit. Med.* 139:17–20.
- Misra, R. C., Kasthuri, D., and Chuttani, H. K., 1966, Adult coeliac disease in tropics, *Br. Med. J.* 2:1230–1232.
- Misra, R. C., Krishnan, N., Ramalingaswami, V., and Chuttani, H. K., 1967, Tropical sprue in Northern India, *Scand. J. Gastroenterol.* 2:193–199.
- Mollin, D. L., and Booth, C. C., 1968, Observations on the pathogenesis of tropical sprue, in: *Tropical Medicine Conference, 1967* (J. H. Walters, ed.), Pitman, London, pp. 96–108.
- Mollin, D. L., and Booth, C. D., 1971, Chronic tropical sprue in London, in: *Tropical Sprue and Megaloblastic Anaemia*, Wellcome Trust Collaborative Study, Wellcome Trust, Churchill Livingstone, Edinburgh, London, pp. 61–127.
- Mollin, D. L., Booth, C. C., and Baker, S. J., 1957, The absorption of vitamin B₁₂ in control subjects, in Addisonian pernicious anaemia and in the malabsorption syndrome, *Br. J. Haematol.* 3:412–428.
- Montgomery, R. D., Beale, D. J., Sammons, H. G., and Schneider, R., 1973, Postinfective malabsorption: A sprue syndrome, *Br. Med. J.* 2:265–268.
- Moshal, M. G., Hift, W., Kallichurum, S., and Pillay, K., 1975, Endemic tropical sprue in Africa, *J. Trop. Med. Hyg.* 78:2–5.
- Nadel, H., and Gardner, F. H., 1956, Bacteriological assay of small bowel secretion in tropical sprue, *Am. J. Trop. Med.* 5:686–689.
- Nasr, K., Haghighi, P., Abadi, P., Lahimgarzadeh, A., Hedayatti, H., Halsted, J. A., and Reinhold,

- J. D., 1976, Idiopathic enteropathy: An evaluation in rural Iran with an appraisal of nutrient loss, *Am. J. Clin. Nutr.* 29:169–176.
- Nelson, R., 1973, Coeliac disease in children of Asian immigrants, *Lancet* 1:348–350.
- O'Brien, W., 1968, Clinical presentation and pathology of tropical sprue, in: *Tropical Medicine Conference (1967)* (J. H. Walters, ed.), Pitman, London, pp. 86–95.
- O'Brien, W., 1971, Historical survey of tropical sprue affecting Europeans in south-east Asia, in: *Tropical Sprue and Megaloblastic Anaemia*, Wellcome Trust Collaborative Study, Wellcome Trust, Churchill Livingstone, Edinburgh, London, pp. 13–23.
- O'Brien, W., and England, N. W. J., 1966, Military tropical sprue from south-east Asia, *Br. Med. J.* 2:1157–1162.
- O'Brien, W., and England, N. W. J., 1971, Tropical sprue amongst British servicemen and their families in south-east Asia, in: *Tropical Sprue and Megaloblastic Anaemia*, Wellcome Trust Collaborative Study, Wellcome Trust, Churchill Livingstone, Edinburgh, London, pp. 25–60.
- Olson, S. W., and Layne, J. A., 1947, Sprue as a sequel to the war's migration of military personnel, *Gastroenterology* 8:221–227.
- Paterson, D. E., and Baker, S. J., 1958, The radiological investigation of the small intestine in tropical idiopathic malabsorption syndrome, *J. Faculty Radiol.* 9:183–194.
- Perea, V. D., Fernan-Zegarra, Cruz, V. M., Ballon, R., and Picoaga, J. L., 1978, Non-specific ileojejunitis in Arequipa, Peru, *Lancet* 2:550–552.
- Pittman, F. E., and Pittman, J. C., 1976, Tropical sprue in American servicemen following return from Vietnam, *Am. J. Dig. Dis.* 21:393–398.
- Platt, B. S., Heard, C. R. C., and Stewart, R. J. C., 1964, The effects of protein–calorie deficiency on the gastrointestinal tract, in: *The Role of the Gastrointestinal Tract in Protein Metabolism* (H. N. Munro, ed.), Blackwell, Oxford, pp. 227–241.
- Rajan, K. T., Rao, P. S. S., Ponnusamy, I., and Baker, S. J., 1961, The oral glucose-tolerance test in tropical malabsorption syndrome, *Br. Med. J.* 1:29–31.
- Reimann, H. A., 1963, Viral dysentery, *Am. J. Med. Sci.* 246:404–409.
- Rhoads, C. P., and Miller, D. K., 1934, Intensive liver extract therapy of sprue, *J.A.M.A.* 103:387–391.
- Rhodes, A. R., Shea, N., and Lindenbaum, J., 1971, Malabsorption in asymptomatic Liberian children, *Am. J. Clin. Nutr.* 24:574–577.
- Rickles, F. R., Klipstein, F. A., Tomasini, J., Corcino, J., and Maldonado, N., 1972, Long-term follow-up antibiotic-treated tropical sprue, *Ann. Intern. Med.* 76:203–210.
- Robins, S. J., Garcia-Palmieri, M., and Rubio, C., 1967, Low serum cholesterol levels and subclinical malabsorption, *Ann. Intern. Med.* 66:556–567.
- Roche, M., and Layrisse, M., 1966, The nature and causes of hookworm anemia, *Am. J. Trop. Med. Hyg.* 15:1032–1093.
- Rodriguez-Molina, R., 1943, Sprue in Puerto Rico: Ten years later, *Puerto Rico J. Public Health Trop. Med.* 18:314–340.
- Rodriguez-Molina, R., Cancio, M., and Asenjo, C. F., 1960, The effect of folic acid on the steatorrhea of tropical sprue and other absorption tests, *Am. J. Trop. Med. Hyg.* 9:308–314.
- Rogers, L., 1938, The use of prontosil in sprue, *Br. Med. J.* 2:943–944.
- Rubini, M. E., Sheehy, T. W., Meroney, W. H., and Louro, J., 1961, Exudative enteropathy. II. Observations in tropical sprue, *J. Lab. Clin. Med.* 58:902–907.
- Russell, P. K., Aziz, M. A., Ahmed, N., Kent, T. H., and Gangarosa, E. J., 1966, Enteritis and gastritis in young asymptomatic Pakistani men, *Am. J. Dig. Dis.* 11:296–306.
- Santiago-Borrero, P. J., Maldonado, N., and Horta, E., 1970, Tropical sprue in children, *J. Pediatr.* 76:470–479.
- Santini, R., Aviles, J., and Sheehy, T. W., 1960, Sucrase activity in the intestinal mucosa of patients with sprue and normal subjects, *Am. J. Dig. Dis.* 5:1059–1062.

- Schenk, E. A., Samloff, I. M., and Klipstein, F. A., 1965, Morphologic characteristics of jejunal biopsy in celiac disease and tropical sprue, *Am. J. Pathol.* 47:765-781.
- Schenk, E. A., Samloff, I. M., and Klipstein, F. A., 1968, Morphology of small bowel biopsies, *Am. J. Clin. Nutr.* 21:944-961.
- Schneider, R. E., Anderson, C., and Shiffman, M., 1974, Prevalence of d-xylose malabsorption in rural communities of Guatemala, Resúmenes, V congreso Mundial de Gastroenterología, Mexico City, p. 311.
- Schneider, R. E., and Viteri, F. E., 1972, Morphological aspects of the duodenojejunal mucosa in protein-calorie malnourished children and during recovery, *Am. J. Clin. Nutr.* 25:1092-1102.
- Schneider, R. E., and Viteri, F. E., 1974, Luminal events of lipid absorption in protein-calorie malnourished children; relationship with nutritional recovery and diarrhea. I. Capacity of the duodenal content to achieve micellar solubilization of lipids, *Am. J. Clin. Nutr.* 27:777-787.
- Sheehy, T. W., and Anderson, P. R., 1965, Disaccharidase activity in normal and diseased small bowel, *Lancet* 2:1-5.
- Sheehy, T. W., and Perez-Santiago, E., 1961, Antibiotic therapy in tropical sprue: The role of dietary folic acid in the hematologic remission associated with oral antibiotic therapy, *Ann. Intern. Med.* 61:721-728.
- Sheehy, T. W., Rubini, M. E., Perez-Santiago, E., Santini, R., and Haddock, J., 1961, The effect of "minute" and "titrated" amounts of folic acid on the megaloblastic anemia of tropical sprue, *Blood* 18:623-636.
- Sheehy, T. W., Meroney, W. H., Cox, R. S., Jr., and Soler, J. E., 1962a, Hookworm disease and malabsorption, *Gastroenterology* 42:148-156.
- Sheehy, T. W., Baggs, B., Perez-Santiago, E., and Floch, M. H., 1962b, Prognosis of tropical sprue; a study of the effect of folic acid on the intestinal aspects of acute and chronic sprue, *Ann. Intern. Med.* 57:892-908.
- Sheehy, T. W., Cohen, W. C., Wallace, D. K., and Legters, L. J., 1965, Tropical sprue in North Americans, *J.A.M.A.* 194:1069-1076.
- Sheehy, T. W., Legters, L. J., and Wallace, D. K., 1968, Tropical jejunitis in Americans serving in Vietnam, *Am. J. Clin. Nutr.* 21:1013-1022.
- Singh, B., Jeejeebhoy, K. N., Samuel, A. M., and Korgaonkar, K. S., 1973, The distribution and degradation of albumin in tropical sprue. I. A comparative analysis of albumin turnover data using different mathematical models, *Indian J. Med. Res.* 61:870-884.
- Smith, M. E., Matty, A. J., and Blair, J. A., 1970, The transport of pteroylglutamic acid across the small intestine of the rat, *Biochim. Biophys. Acta* 219:37-46.
- Sparberg, M., Knudson, K. B., and Frank, S., 1967, Tropical sprue from the Philippines: Report of three cases, *Milit. Med.* 132:809-815.
- Spies, T. D., and Suarez, R. M., 1948, Response of tropical sprue to vitamin B₁₂, *Blood* 3:1213-1220.
- Spies, T. D., Milanes, F., Menendez, A., Koch, M. B., and Minnich, V., 1946, Observations on treatment of tropical sprue with folic acid, *J. Lab. Clin. Med.* 31:227-241.
- Sprinz, H., Sribhibhadh, R., Gangarosa, E. J., Benyajati, C., Kundel, D., and Halstead, S., 1962, Biopsy of small bowel of Thai people: With special reference to recovery from Asiatic cholera and to an intestinal malabsorption syndrome, *Am. J. Clin. Pathol.* 38:43-51.
- Stanfield, J. P., Hutt, M. S. R., and Tunnicliffe, R., 1965, Intestinal biopsy in kwashiorkor, *Lancet* 2:519-523.
- Stefanini, M., 1948, Clinical features and pathogenesis of tropical sprue: Observations on a series of cases among Italian prisoners of war in India, *Medicine (Baltimore)* 27:379-427.
- Stemmermann, G. N., 1967, Strongyloidiasis in migrants. Pathological and clinical considerations, *Gastroenterology* 53:59-70.

- Suarez, R. M., 1931, El tratamiento del esprú y el uso del extracto acuoso de hígado en su anemia, *Bol. Asoc. Med. P.R.* 23:74–86.
- Suarez, R. M., 1938, Clinical and hematologic review of sprue based on the study of 150 cases, *Ann. Intern. Med.* 12:529–535.
- Svoboda, D., Grady, H., and Higginson, J., 1966, The effects of chronic protein deficiency in rats. II. Biochemical and ultrastructural changes, *Lab. Invest.* 15:731–749.
- Swaminathan, N., Mathan, V. I., Baker, S. J., Radhakrishnan, A. N., 1970, Disaccharidase levels in jejunal biopsy specimens from American and south Indian control subjects and patients with tropical sprue, *Clin. Chim. Acta* 30:707–712.
- Swanson, V. L., and Thomassen, R. W., 1965, Pathology of the jejunal mucosa in tropical sprue, *Am. J. Pathol.* 46:511–551.
- Swanson, V. L., Wheby, M. S., and Bayless, T. M., 1966, Morphologic effects of folic acid and vitamin B₁₂ on the jejunal lesion of tropical sprue, *Am. J. Pathol.* 49:167–191.
- Tandon, B. N., Das, B. C., Saraya, A. K., and Deo, M. G., 1966a, Functional and structural studies of small bowel in ankylostomiasis, *Br. Med. J.* 1:714–716.
- Tandon, B. N., Iyenger, K. P., Deo, M. G., and Saraya, A. K., 1966b, A study of sprue syndrome in adults in Northern India, *J. Assoc. Physicians India* 14:197–202.
- Tandon, B. N., Magotra, M. L., Saraya, A. K., and Ramalingaswami, V., 1968, Small intestine in protein malnutrition, *Am. J. Clin. Nutr.* 21:813–819.
- Tandon, B. N., Newberne, P. M., and Young, V. R., 1969, A histochemical study of enzymes and ultrastructure of the jejunal mucosa of protein-depleted rats, *J. Nutr.* 99:519–530.
- Tandon, B. N., Bose, S. L., and Saikia, B., 1974, An evaluation of prednisolone, tetracycline and high protein diet for the therapy of idiopathic tropical malabsorption syndrome, *Indian J. Med. Res.* 62:704–711.
- Thin, G., 1897, *Psilosis or Sprue*, 2nd ed., Churchill, London.
- Thomas, G., and Clain, D. J., 1976, Endemic tropical sprue in Rhodesia, *Gut* 17:877–887.
- Thomas, G., Clain, D. J., and Wicks, A. C. B., 1976, Tropical enteropathy in Rhodesia, *Gut* 17:888–894.
- Tomkins, A. M., Drasar, B. S., and James W. P. T., 1975, Bacterial colonisation of jejunal mucosa in acute tropical sprue, *Lancet* 1:59–62.
- Tomkins, A. M., Smith, T., and Wright, S. G., 1978, Assessment of early and delayed responses in vitamin B₁₂ absorption during antibiotic therapy in tropical malabsorption, *Clin. Sci. Mol. Med.* 55:533–539.
- Troncale, F. J., Keusch, G. T., Miller, L. H., Olsson, R. A., and Buchanan, R. D., 1967, Normal absorption in Thai subjects with non-specific jejunal abnormalities, *Br. Med. J.* 4:578–580.
- Vaish, S. K., Sampathkumar, J., Jacob, R., and Baker, S. J., 1965, The stomach in tropical sprue, *Gut* 6:458–465.
- Veeger, W., Thije, O. J. Ten., Hellemans, N., Mandema, E., and Niewig, H. O., 1965, Sprue with a characteristic lesion of the small intestine associated with folic deficiency, *Acta Med. Scand.* 177:493–502.
- Walia, B. N. S., Sidhu, J. K., Tandon, B. N. Ghai, O. P., and Bhargava, S., 1966, Coeliac disease in North Indian children, *Br. Med. J.* 2:1233–1234.
- Walker-Smith, J. A., and Reye, R. D. K., 1971, Small intestinal morphology in Aboriginal children, *Aust. N.Z. J. Med.* 4:377–384.
- Walters, J. H., 1947, Dietetic deficiency syndromes in Indian soldiers, *Lancet* 1:861–865.
- Webb, J., 1956, *Tropical Sprue in Hong Kong*, M.D. Thesis, Durham University, Durham, England.
- Whalen, G. E., Rosenberg, E. D., Strickland, G. T., Gutman, R. E., Cross, J. H., Watten, R. H., Uylangco, C., and Dizon, J. J., 1969, Intestinal capillariasis. A new disease in man, *Lancet* 1:13–16.

- Wheby, M. S., and Bayless, T. M., 1968, Intrinsic factor in tropical sprue, *Blood* 31:817–820.
- Wheby, M. S., Swanson, V., and Bayless, T. M., 1968, Jejunal crypt cell and marrow morphology in tropical sprue, *Ann. Intern. Med.* 69:427–434.
- Wheby, M. S., Swanson, V. L., and Bayless, T. M., 1971, Comparison of ileal and jejunal biopsies in tropical sprue, *Am. J. Clin. Nutr.* 24:117–123.
- Yardley, J. H., Takano, J., and Hendrix, T. R., 1964, Epithelial and other mucosal lesions of the jejunum in giardiasis. Jejunal biopsy studies, *Bull. Johns Hopkins Hosp.* 115:389–406.

Tropical Splenomegaly

NABEEN C. NAYAK

1. AN ENTITY OR A SYNDROME?

Diseases associated with conspicuous splenomegaly have frequently been encountered in tropical and subtropical countries. Although many of these are caused by infections including malaria and kala azar, several not belonging to the category have been referred to as “tropical splenomegaly” (Marsden *et al.*, 1965). The name is inappropriate since not only is the condition also seen, sometimes in impressive numbers, in temperate regions, but splenomegaly appears to be only one of its important features. Identical or very similar disorders have been described under a host of names including splenic anemia (Banti, 1883; Osler, 1900), hepatolental fibrosis (McMichael, 1934), Bengal splenomegaly (De, 1932; De and Tribedi, 1939), cryptogenic splenomegaly (Cook *et al.*, 1963), idiopathic tropical splenomegaly (Williams *et al.*, 1966), idiopathic presinusoidal portal hypertension (Polish *et al.*, 1962), idiopathic portal hypertension (Boyer *et al.*, 1967; Sugiura and Ichihara, 1971; Kobayashi *et al.*, 1976), hepatoportal sclerosis (Mikkelsen *et al.*, 1965), noncirrhotic portal fibrosis with portal hypertension (Basu *et al.*, 1967; Basu Mallick *et al.*, 1967; Basu and Guharay, 1978), noncirrhotic portal fibrosis (Indian Council of Medical Research, 1969; Sama *et al.*, 1971), big spleen disease (Leather, 1961; Hamilton *et al.*, 1967), noncirrhotic portal hypertension (Marleau *et al.*, 1975), occlusive venopathy of the liver (Nayak and Ramalingaswami, 1969; Iber, 1969), essential portal hypertension (Benhamou *et al.*, 1962), primary portal hypertension (Bhalerao *et al.*, 1968), and idiopathic noncirrhotic portal hypertension (Futagawa *et al.*, 1980).

In spite of the confusion inherent in such widely varied nomenclature, it has

become clear through the years that the principal manifestations of this syndrome are prominent splenomegaly, repeated gastrointestinal bleeding, and anemia, all three resulting from sustained and high portal hypertension. There is no accompanying cirrhosis or any obvious obstruction to the extrahepatic portion of the porto-splenic venous system. For long it was considered that the syndrome is an expression of diseases of diverse nature, until studies on large series of patients from India and Japan as well as on smaller numbers from other areas indicated that this form of portal hypertension appears not only to be frequent in several countries but also to constitute a distinct, identifiable entity. Although universal agreement on a single nomenclature is yet to be attained, the International Association for the Study of the Liver, in their standardization of nomenclature (Leevy, Popper, and Sherlock, 1977), accepted the disease as a specific entity and adopted the term hepatoportal sclerosis, with the synonyms obliterative portal venopathy of the liver, hepatolienal fibrosis, and idiopathic portal hypertension. The disease appears to have generally well-defined clinical, functional, radiological, hemodynamic, and morphological criteria.

2. EXTENT OF THE PROBLEM

Having a protracted course and manifested chiefly with features of portal hypertension, the disease seems to be widely prevalent in parts of southeast Asia (Indian Council of Medical Research, 1969; Cook *et al.*, 1963; Boyer *et al.*, 1967), Africa (Leather, 1961; Marsden *et al.*, 1965), and Japan (Okuda, 1979b). Smaller numbers of cases are encountered from time to time in other regions as well. The condition is widespread throughout India (Basu *et al.*, 1967; Indian Council of Medical Research, 1969; Sama *et al.*, 1971; Aikat *et al.*, 1979; Basu, 1980) and in Japan (Imanaga *et al.*, 1962; Sugiura and Ichihara, 1971; Kobayashi *et al.*, 1976). An estimated one-fourth of all patients presenting to the hospital with portal hypertension in India and Japan suffer from this disease (Sama *et al.*, 1971; Basu and Guharay, 1978; Aikat *et al.*, 1979; Imanaga *et al.*, 1962), whereas in the West this figure is as low as 3% to 5% (Iber, 1969). Because of the high degree of morbidity and repeated, although generally nonfatal, gastrointestinal bleeding, high incidence rates in countries such as India and Japan pose a great concern for the medical care of such patients.

3. ESSENTIAL FEATURES

3.1. Epidemiology

Irrespective of the geographic distribution, the disease predominantly affects individuals in the third and fourth decades of life, with its peak incidence

around 35 to 40 years of age. Ages of affected individuals range from less than 10 to 65 years, although the major part of the distribution curve seems to lie between the middle of the second decade and the end of the fifth decade (Table I). This curve is significantly different from those of the two other major causes of portal hypertension, cirrhosis and extrahepatic porto-systemic obstruction. In the latter, much younger individuals are affected, the peak incidence coming between 10 and 15 years of age, and only rarely are individuals above the age of 35 years affected. The age distribution pattern in cirrhosis seems to be quite different from that of tropical splenomegaly in western countries as well as in Japan in the sense that the majority of individuals with cirrhosis are between 25 and 70 years of age, with the peak incidence appearing around 50 years. In India, on the other hand, there seems to be some degree of overlapping in the age distributions of these two conditions, although the curve of cirrhosis incidence still lies somewhat to the right of that for tropical splenomegaly, and it is rare to encounter cirrhosis in individuals between the ages of 5 and 20 years. The figures for tropical splenomegaly, particularly in India, may be influenced by the fact that patients whose predominant symptom is bleeding tend to report to the hospital earlier than those with splenomegaly as the major manifestation.

The sex distribution seems to be different in the large series reported from India and Japan (Table I). Among Indian patients, males distinctly outnumber females in a ratio between 2 and 3.5; in Japan, on the other hand, the number of female patients is approximately three times that of males (Okuda, 1979b). This difference in the sex distribution between the two countries is currently difficult to explain, although it is possible that a proportion of the male excess in India may depend on the sociocultural environment and the larger proportion of male admissions to most hospitals (Sama *et al.*, 1971). There is no difference in seasonal or annual incidence of the disease (Okuda, 1979b), and a trend for general high incidence is noted among individuals living in warm climates and those belonging to the lower middle income groups.

Table I
Age, Sex, and Important Presenting Features in Patients with Tropical
Splenomegaly

	Number of reported cases (8 years)	Age (years) (range and mean)	Male : female ratio	Gross splenomegaly	Conspicuous GI bleeding
India	293	10–60 32	2.0	98%	65%
Japan	624	10–70 38	0.3	91%	38%

It is interesting that the poorest class of people in whom protein malnutrition and associated complications are most common do not show a high incidence of this condition. The distribution patterns among urban and rural populations are equivocal. Although in Japan the disease is more prevalent in the countryside (Okuda, 1979b), in India, the largest proportion of the patient population has been reported to be city dwellers (Basu and Guharay, 1978). There is no specific occupation that has been found to make an individual more prone to get the disease, although among Japanese women patients, approximately half are housewives.

Associations indicating infections by malarial parasites, leishmanias, schistosomes, and hepatitis viruses as well as evidence of continued exposure to vinyl chloride, arsenic, or consumption of alcohol are uniformly lacking.

3.2. Clinical Manifestations

The disease principally manifests with symptoms and signs related to protracted and high portal hypertension, generally unaccompanied by any feature of hepatocellular failure. Most patients at their first appearance in the hospital give several years' history of the disease identified either by themselves or by a physician during examination for minor ailments (Sama *et al.*, 1971). The two most predominant symptoms are splenomegaly and gastrointestinal bleeding (Table I), the latter commonly in the form of large bouts of hematemesis. The frequencies of these two primary and important manifestations, however, vary depending on whether a physician or a surgeon is reporting the series. In case of the former, the relative frequencies of splenomegaly and hematemesis have been approximately 100% and 50%, respectively, whereas in the case of the latter these are 93% and 70%. On the other hand, when a careful history is sought, a remarkably uniform initial symptom of splenomegaly can be elicited in almost all of the patients. The spleen is very large, measuring between 2 and 27 cm below the costal margin, and was first observed an average 7 years attendance (Sama *et al.*, 1971; Basu and Guharay, 1978). When very large series are analyzed, splenomegaly emerges as the single most important clinical presentation (91%), and gastrointestinal bleeding manifests in approximately 40% of patients (Okuda, 1979b).

Gastrointestinal bleeding is chiefly observed in the form of hematemesis, and melena is relatively rare. Hematemesis is mostly recurrent, averaging 3.5 episodes prior to hospital admission (Sama *et al.*, 1971; Basu and Guharay, 1978), and in more than half of the patients each bout of bleeding may be severe. Interestingly, however, these massive and repeated bleeds are tolerated very well without the patient developing any evidence of hepatocellular failure such as encephalopathy, jaundice, or rapidly developing ascites. This seems to be an important pointer in differentiating the condition from the more fatal form of

liver disease, cirrhosis. The third important clinical abnormality in these patients is anemia which results partly from a functional hypersplenism and partly from the repeated bleeding episodes. A mild hepatomegaly may be seen in a proportion of patients, and engorged abdominal veins reflect the severity and duration of portal hypertension. Minimal to moderate ascites may be seen in some patients, particularly in those who have had several episodes of bleeding. Features of hepatocellular failure such as gynecomastia, palmar erythema, jaundice, and spider angioma are, however, exceedingly uncommon.

3.3. Radiological Findings

Esophageal varices, as the most important radiological evidence of portal hypertension, are evident in the majority (71% to 100%) of patients (Sama *et al.*, 1971; Basu and Guharay, 1978). Radiological maneuvers to outline the portal venous system including spleno-porto venography, umbilical vein catheterization and venography, transhepatic portal venography, and intraoperative portal venography have all revealed wide and prominent anastomotic channels between the portal venous system and the systemic veins. Regarding visualization of the intrahepatic vascular channels, although there is some degree of variability among individual cases of tropical splenomegaly, the appearances of the vasculature in this condition are distinctly different from those in cirrhosis. Paucity of medium-sized portal vein branches, irregular division of the peripheral radicals, abrupt interruptions in the dye in some of the vessels (Figure 1), an avascular zone in the subcapsular area of the liver, nonvisualization of some of the large intrahepatic portal vein branches, and appearances suggestive of multichanneled

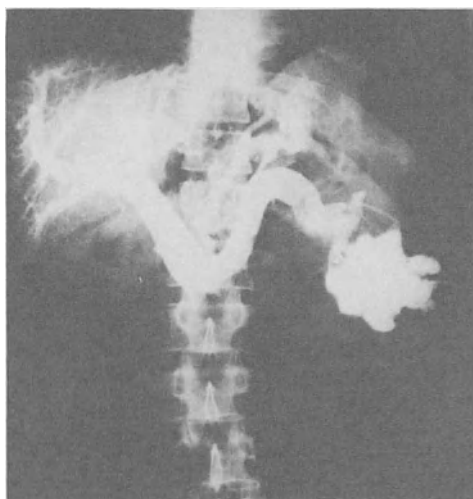


Figure 1. Splenoportovenogram following intrasplenic injection of dye in a patient with idiopathic splenomegaly showing abnormalities in the intrahepatic portal vein branches. Some are indistinctly visualized, whereas others present a "cut-off" or "withered-tree" appearance.

cavernomatous formation in the hilar and more central part of the liver are prominent radiological features of this disease (Futagawa *et al.*, 1980; Sama *et al.*, 1971; Basu and Guharay, 1978). Truncation of the major branches of the portal vein, a disparity between the large and medium-sized intrahepatic branches, and poor or nonvisualization of the peripheral radicles impart what has been referred to as “cut-off” or “withered-tree” patterns (Basu and Guharay, 1978).

The radiological appearances described above are different from those seen in patients with cirrhosis in whom sweeping curves of the vessels indicating nodule formation are clearly apparent. A proper portal venograph is an essential requirement in the diagnosis of the condition, since it not only helps to differentiate the disease from cirrhosis but would also exclude the possibility of an extrahepatic porto-splenic venous block as the major cause of portal hypertension.

3.4. Hemodynamic Data

Information obtained from these investigations indicates that the portal hypertension in such patients is related to a presinusoidal resistance to intrahepatic blood flow. Portal vein pressure (PVP) obtained by various techniques such as intrasplenic pulp pressure measurement, transhepatic portal vein catheterization, or intraoperative direct measurement reveals very high values averaging 300 mm of water (Sakata *et al.*, 1979; Sama *et al.*, 1971; Basu and Guharay, 1978). This heightened pressure is comparable to that seen in patients with cirrhosis. Wedged hepatic venous pressure (WHVP), which indicates postsinusoidal resistance, is only mildly elevated (average 150 mm of water) and is normal in some patients, although the mean values are significantly higher than in controls. The increase

Table II
Hemodynamic Data in Tropical Splenomegaly and Related Diseases

	Tropical splenomegaly	Cirrhosis	Extrahepatic porto-splenic obstruction
Portal vein pressure (PVP)	High	High	High (proximal to obstruction)
Wedged hepatic vein pressure (WHVP)	Slight to moderately high or normal	Very high	Normal
PVP-WHVP gradient	Moderately high	Low	High
Estimated hepatic blood flow	Normal	Low	Normal

in WHVP, on the other hand, is distinctly less than that seen in cirrhosis, and thus, the gradient between PVP and WHVP is much steeper in patients with tropical splenomegaly than in those with cirrhosis. The slight increase in WHVP is possibly a result of the variable and widespread fibrosis in the liver, although unlike cirrhosis, the heightened PVP is caused largely by presinusoidal resistance. The estimated hepatic blood flow (EHBF) evaluated by various techniques indicates that in tropical splenomegaly the flow is no different from that in controls, and is unlike that seen in cirrhosis, where values are indeed quite low. Thus, a high PVP, a moderate elevation of WHVP, a steep gradient between PVP and WHVP, and a relatively normal EHBF are characteristics of the hemodynamic aspects of tropical splenomegaly (Table II).

3.5. Natural History

The disease generally takes a benign course, patients living with the disease for several years before either repeated massive hematemesis or a large spleen presenting as a conspicuous intraabdominal lump brings them to the hospital. Tolerance of the bleeding episodes and of anemia is remarkably good, only a very small proportion of chronically ill patients showing signs of hepatocellular failure following the bleed. Recovery from the temporary incapacitation brought about by the bleed is either spontaneous or rapid following resuscitative measures in the hospital. Some investigators believe that with minimal medical management these patients have an extremely good prognosis and live their normal span of life. Others, however, are of the opinion that in countries where medical facilities are not readily available within immediate reach of the patient, repeated bleeders may be significantly benefited by a proper shunt surgery. Liver function tests are almost always within normal limits, a feature that is relevance in the clinical differentiation of this condition from cirrhosis. In a minority of individuals there is a slow but progressive deterioration of liver functions, possibly because of the hepatic effects of repeated bleeds. Cases are on record in which female patients with marked splenomegaly or with bouts of bleeding have gone through the stresses of repeated pregnancy and parturition without significant deterioration in the clinical course of disease.

4. PATHOLOGICAL ASPECTS

Structural changes in the liver in tropical splenomegaly are in some aspects highly characteristic, whereas in others they are quite nonspecific. When examined in detail, particularly on autopsy and operative wedge biopsy material, no patient shows an entirely normal liver. It is important to realize this because portal hypertension "in the presence of normal liver" (Iber, 1970) or "in the

absence of liver disease'' (Miller and Brandt, 1962) must be viewed in the proper perspective. It is only possibly in needle biopsy material for minimal or no changes to be observed and when it becomes important for the pathologist to indicate to the clinician that evidence of cirrhosis is lacking in an otherwise adequate specimen. Even though, during the initial reports on large series of cases, the characteristic pathological features of the disease were not clearly identified (Basu and Aikat, 1963; Basu *et al.*, 1967; Imanaga *et al.*, 1962), more meticulous and comparative studies starting with those of Nayak and Ramalingaswami (1969) and Mikkelsen *et al.* (1965) and followed later by others (Mukherjee *et al.*, 1979; Aikat *et al.*, 1979; Aida and Okudaira, 1979a,b; Kage *et al.*, 1979a,b; Koga *et al.*, 1979) clearly revealed not only that the changes in the liver in this disease are consistent and uniform within a relatively broad spectrum but also that the pathological alterations seem to be similar in disease occurring in different parts of the world (Okuda, 1979a; Kage *et al.*, 1979b; Futagawa *et al.*, 1980). By far the outstanding morphological markers of the disease seem to be portal fibrosis and obliterative vascular changes involving the intrahepatic portal radicles and the sequelae of these abnormalities.

4.1. Hepatic Alterations

4.1.1. Macroscopic Appearance

The liver weight is not particularly abnormal. The range extends from somewhat shrunken organs to those that are 10 to 25% heavier than normal. The shape of the organ, on the other hand, is very frequently distorted, with non-uniform appearance of the surface, some areas appearing smooth and others wrinkled, furrowed, or even indistinctly nodular. Focal areas and streaks of opacification of the capsule are common. On occasion the two main lobes are disproportionate in size and shape, with one of the lobes relatively atrophied although the other shows compensatory hypertrophy. It is the left lobe that is more frequently involved by the atrophic process than the right. There is no significant change in the color of the organ, although the distribution and thickness of the scar tend to impart a varied look to the surface. Uniformly distributed nodules are consistently lacking, but on occasion a few medium-sized or even large regenerating nodules may be apparent on the surface. This is particularly applicable to the inferior surface of the liver, a feature that should be taken note of by the operating surgeon who, on viewing this particular part of the organ at operation, may mistakenly consider the liver to be cirrhotic. Irregular scars, sometimes deep and radiating, frequently cause an irregularly bossilated appearance of the liver surface. In general, the organ is firmer than normal, and the edges, particularly the inferior margin, are somewhat rounded.

On a cut surface, irregular fibrosis almost exclusively involving the portal



Figure 2. A slice of liver showing a somewhat irregular and wrinkled surface and variable portal fibrosis on the cut surface. In one area there is excessive perivascular fibrosis (arrow).

tracts and absence of diffuse nodularity are the most conspicuous features (Figure 2). Slices of the liver may present not only great variability in the appearance of the parenchyma among individual cases but also from one part to another within the same liver. Almost completely smooth-appearing parenchyma with minimal stellate or rounded scars to several intercommunicating fibrous septa isolating islands of liver tissue in some areas but not in others are recognized features of the disease. In some cases, the fibrosis seems to be more prominent in the hilar area, with perivenular sclerosis that extends along the main branches of the blood vessels into the deeper parenchyma (Figure 2). On rare occasions the main intrahepatic branches of the portal vein are narrowed, and organizing mural or luminal thrombi can be seen in some. The portal vein branches in the middle and peripheral part of the liver are generally inconspicuous because of fibrosis around the blood vessels in the portal tracts. In brief, the most significant macroscopic changes include irregular scarring, parenchymal distortion not amounting to the diffuse nodularity of cirrhosis, capsular thickening in focal areas, and intrahepatic portal venous abnormalities.

4.1.2. Microscopic Features

As expected from the macroscopic features, the principal changes at the light microscopic level are irregular fibrosis and blood vascular changes. The fibrosis is primarily in the portal tract but can be greatly variable in degree, extent, and location. The fibrosed portal tracts may prominently connect one another, enclosing irregular islands of liver cell plates and, in small and unrepresentative specimens, may give a false impression of cirrhosis. The fibrosed areas may have different contours, often multiprocessed stellate type (Figure 3), some-

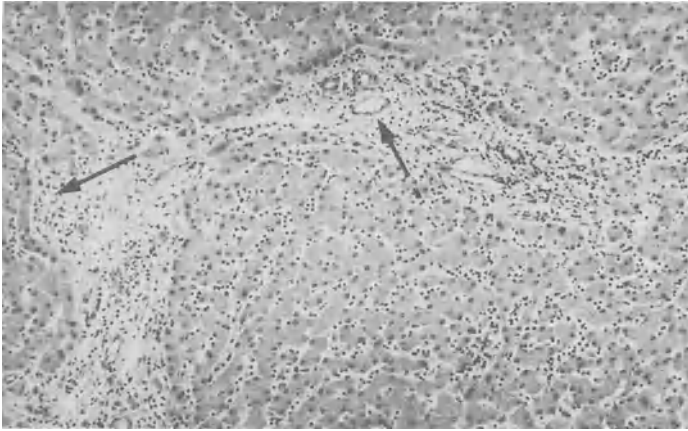


Figure 3. Irregular stellate fibrosis of the portal tracts. The portal vein branches are small and inconspicuous (arrow).

times variable-width bands of branching scars (Figure 4), and on other occasions they can be rounded and nodular (Figure 5). The fibrosis generally tends to be more pronounced in the subcapsular area, so that superficial wedge biopsies, however wide, have the distinct disadvantage of posing problems in accurate assessment and differentiation from cirrhosis (Figures 6 and 7). Since the deeper part of the parenchyma is likely to be free of excessive fibrosis, deep narrow wedges need to be examined for clearer interpretation of the hepatic alterations. The scars are mostly bland, without much inflammation (Figures 4–6), but on some occasions, significant numbers of lymphocytes and histiocytes may be encountered (Figure 3). However, pronounced inflammation is not a feature of this disease.

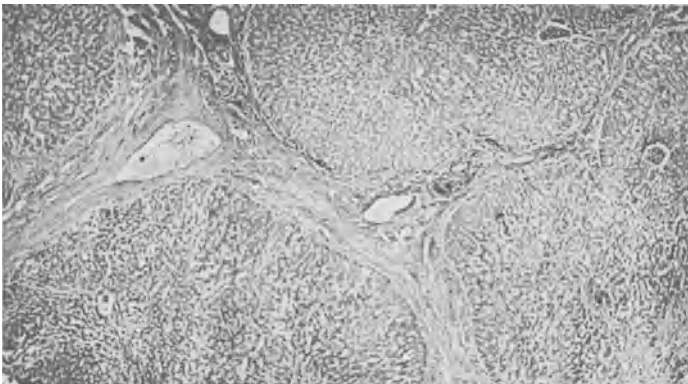


Figure 4. Broad, branching bands of fibrosis involving portal areas and connecting with each other.

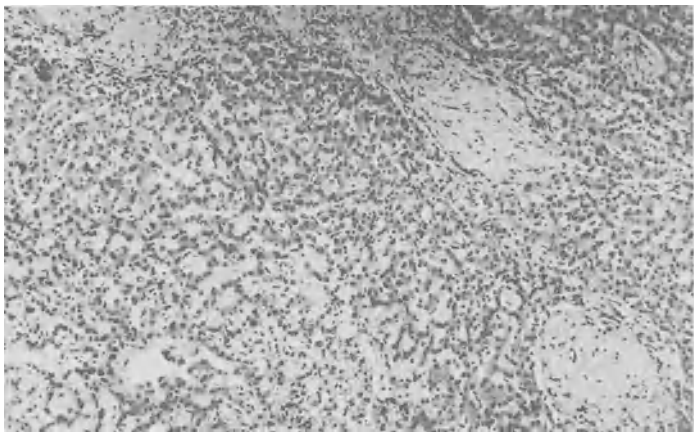


Figure 5. Rounded, sclerosed scars in the portal tracts. Note the inconspicuous portal vein branches in these scars and a normal-appearing central vein in the lower left corner.

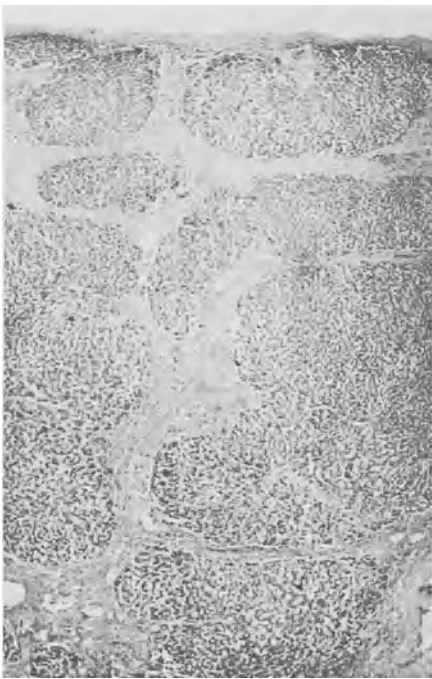


Figure 6. A wedge biopsy specimen of the liver showing marked subcapsular fibrosis isolating nodules of parenchyma in the superficial areas. The resemblance to cirrhosis is apparent.

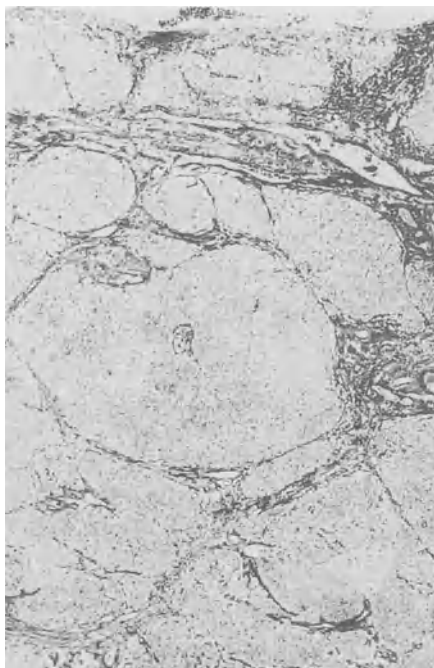


Figure 7. Reticulin stain of another wedge biopsy of liver showing marked areas of portal fibrosis communicating with each other.

Vascular alterations predominantly affect the portal venous system, although the hepatic venous tributaries and intrahepatic branches of the hepatic artery may also reveal changes, in that order of priority. The small portal vein radicles at the periphery of the hepatic lobule (center of the acinus) as well as some of the fourth- to sixth-order divisions of the two main intrahepatic portal vein branches become inconspicuous, smaller, and on occasions obliterated (Figure 3). Unlike normal livers in which the portal vein branches are extremely thin walled, wide lumened, and prominent in comparison to the accompanying hepatic artery branches, in livers of patients with tropical splenomegaly these veins as seen in the frequently fibrosed portal tracts are much smaller, multichanneled, and inconspicuous (Figures 3 and 8). Some of the relatively larger ones among these may show fibrous or muscular thickening of the wall characteristic of the bigger radicles as described below. Often one may find the normal widely patent portal vein branch replaced by a sieve or minute channels distributed in a fibrosed or edematous portal tract (Figure 8). In other areas, such veins may be dilated.

It is this apparently contrasting picture of narrowing and dilatation that may be confusing and has given rise, in the past, to controversies not only in the interpretation of the vascular changes but also in the postulation of the etiopathogenesis of the disease. The most characteristic vascular change of tropi-

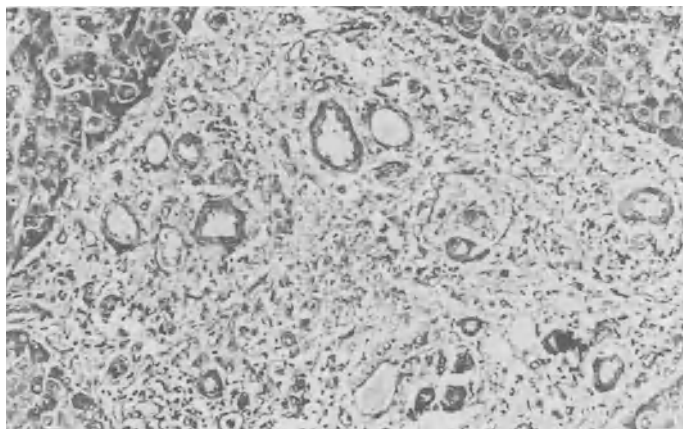


Figure 8. Fibrosed and widened small portal tract showing replacement of single relatively large portal vein by multiple small channels.

cal splenomegaly occurs in the medium-sized portal vein branches and consists of a pronounced but often irregular thickening of the intima comprising transversely running collagen fibers, fibroblasts, and not infrequently smooth muscle fibers (Figures 9 and 10).

Occasionally, some of the smaller lobular branches and, more often, larger intrahepatic portal vein radicles also exhibit these alterations. The change, however, is essentially patchy, involving some parts of the liver and not others, only

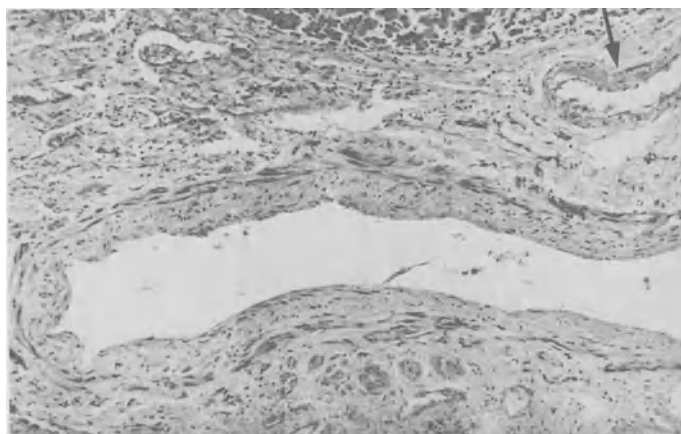


Figure 9. A medium-sized portal vein branch showing pronounced thickening of the wall affecting the intima. The original thin muscle coat can be seen on the outside. The vein wall is several times thicker than the wall of the accompanying artery (arrow).

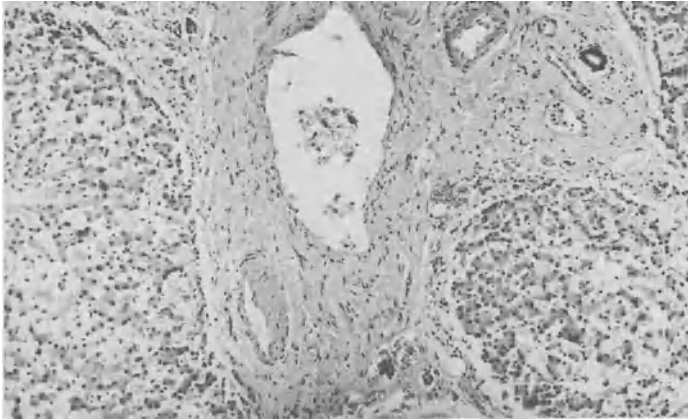


Figure 10. Marked irregular thickening of the portal vein radicle in a medium-sized portal tract. Also note some hypertrophy of the wall of one of the hepatic artery branches.

some vessels in one area, and also certain segments in a vessel and not the adjacent ones (Figure 11). This peculiar nature of involvement by itself has necessitated adequate and wide sampling of liver tissue for examination in order to locate and appreciate them. Obviously, the changes are best seen in properly sampled autopsy material, sometimes seen in deep operative wedge biopsies, and almost never encountered in percutaneous needle biopsy specimens. On careful examination of autopsy specimens, these changes have not only been confirmed in almost all cases of tropical splenomegaly (Nayak and Ramalingaswami, 1969;

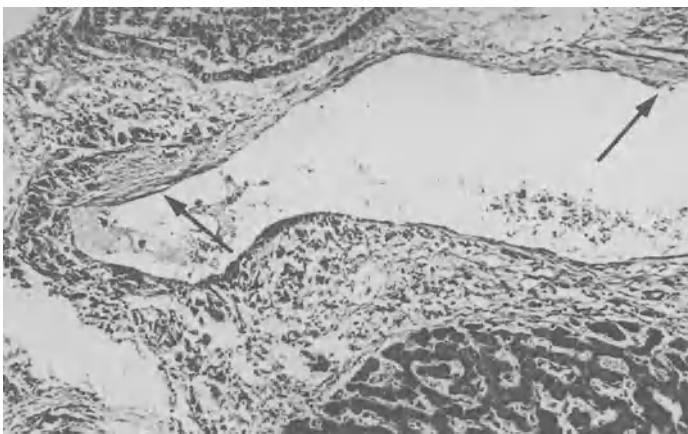


Figure 11. Irregular thickening of the wall of a large intrahepatic portal vein branch. Parts of the wall are markedly thickened (arrows), whereas others are thin and normal.

Mukherjee *et al.*, 1979; Aikat *et al.*, 1979; Aida and Okudaira, 1979a,b; Koga *et al.*, 1979) but have also been found after plastic injection and corrosion of such livers (Boyer *et al.*, 1974) and in portographic studies (Futagawa *et al.*, 1980). The vein wall may become so pronouncedly thick that the vein-to-artery wall thickness ratio instead of being approximately 0.2 may go up as high as 3.0 (Figures 9 and 10). In the neighborhood of these thick-walled veins one frequently sees excessive collagenization and also a variable number of thin-walled venous and lymphatic channels.

The larger portal vein branches of the second and third order often show irregular mural thrombi (Figure 11) which may be recanalized or markedly fibrosed together with a large number of thin-walled veins in the surrounding area, imparting a distinct cavernomatous (so-called "angiomatous") appearance (Figure 12). In fact, in a recent study confined to autopsy material, it has been shown that as many as 90% of cases may show these changes (Mukherjee *et al.*, 1979). Abnormalities in the hepatic vein tributaries are relatively infrequent, although in some areas they can be prominent. Collagenous thickening of the perivenous tissue but without intimal fibrosis as observed in venoocclusive disease is encountered. Sometimes these collagenized veins connect with each other or with adjacent portal areas by fibrous septa. On other occasions, the hepatic veins, particularly the smaller ones, appear dilated.

Branches of the hepatic artery generally show no change, but on rare occasions these may appear somewhat hypertrophic (Figure 10), a phenomenon that is also observed in livers from patients who have extrahepatic porto-splenic venous obstruction.

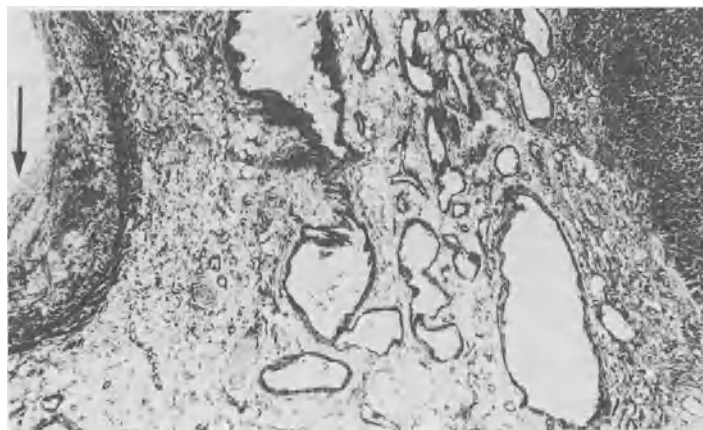


Figure 12. Large portal tract near the hilum of the liver shows mural thrombus incorporated into the wall of a portal vein branch (arrow) and multiple irregular venous channels (cavernomatous change) in the neighborhood.

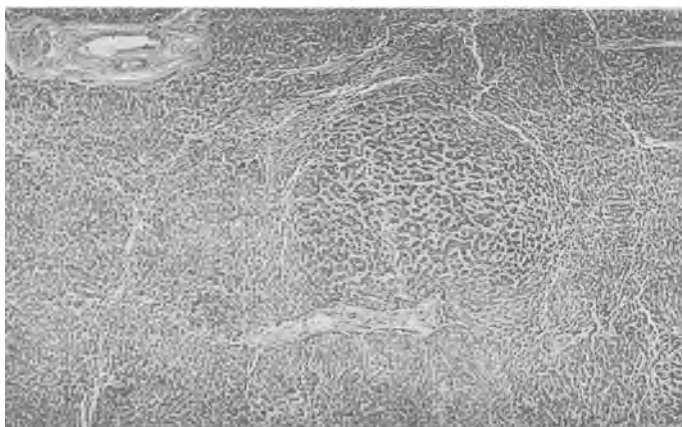


Figure 13. A hyperplastic nodule in the liver comprised of double cell plates but not surrounded by fibrous septa. The neighboring cells are compressed.

The parenchymal tissue itself reveals only minimal and insignificant changes. The most prominent among these are irregular areas of regeneration, sometimes assuming poorly defined nodular forms manifested by double cell plates with nuclei at the periphery and a compression effect on the neighboring parenchyma (Figure 13). These nodules, however, unlike those in cirrhotic livers, are not surrounded by fibrous septa. Focal areas of necrosis, on occasion extending and connecting with each other, are particularly evident in patients who die either after shunt surgery or following some other complication. Parenchymal inflammation is almost totally absent, and other than mild fatty

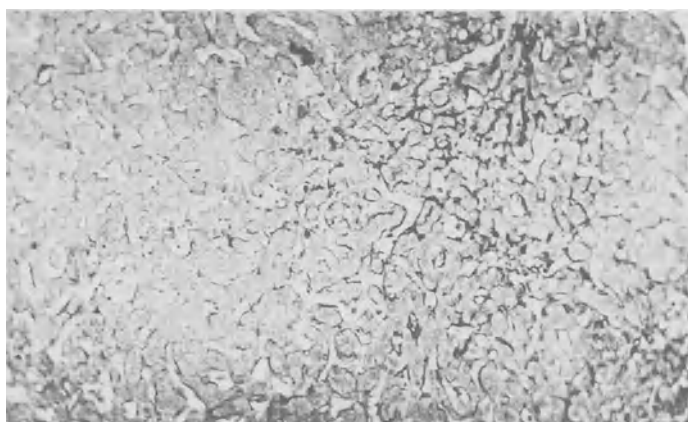


Figure 14. Collagen fibers stained positively by Masson's trichrome stain are seen in the perisinusoidal area.

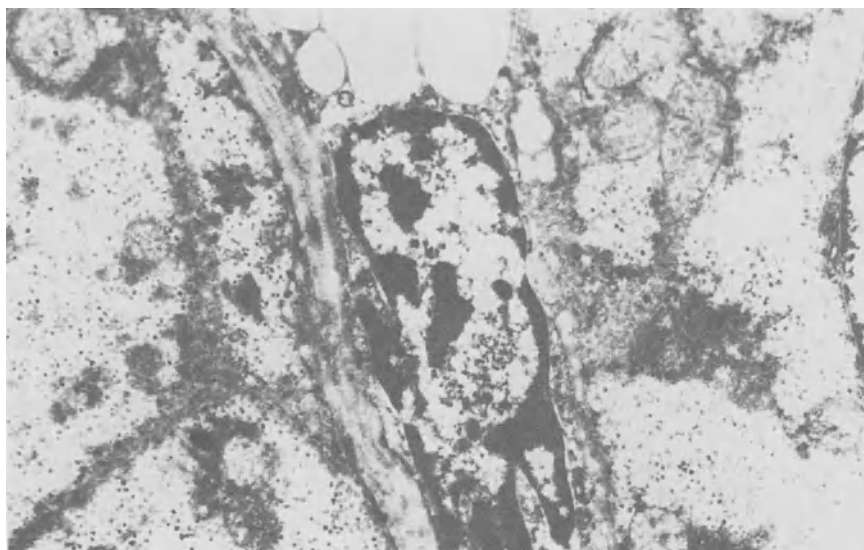


Figure 15. Collagen fibers seen in the perisinusoidal space of Disse on ultrastructural examination of the liver.

changes the liver cells remain generally unremarkable. In a proportion of patients, thin fibers staining positively for collagen are seen patchily in the perisinusoidal areas (Figure 14). This appears to be more clearly brought out on ultrastructural examination (Tandon *et al.*, 1970; Sama *et al.*, 1971) (Figure 15). On the other hand, creeping fibrosis of the type observed in certain other liver diseases is not seen. Endothelial or Kupffer cell hyperplasia is not observed.

4.2. Extrahepatic Portal Venous System

The spectrum of changes in these vessels spreads from dilatation of otherwise uninvolved veins at one end to a thin, cordlike appearance with an extremely narrow lumen at the other. In between these two extremes are seen patchy areas of thickening in the intima, organized or fresh mural thrombi, and irregular fibrous thickening of the wall (Figure 16). Dissemination of pathological changes in the portal, splenic, and superior and inferior mesenteric veins is seen in a small proportion of cases.

4.3. Spleen

The spleen is generally very large, weighing 800 g on average (range 200 to 1500 g), and shows features typical of chronic venous congestion. In addition,



Figure 16. Section of superior mesenteric vein from a patient with tropical splenomegaly. A prominent segmental mural thrombus and irregular thickening of the wall are apparent.

sinus-lining cell hyperplasia, mild fibrosis of the pulp cord, and some plasma cell infiltrations are also frequent features (Nayak and Ramalingaswami, 1969; Aikat *et al.*, 1979; Nakagawa and Kajihara, 1979a). Fibrous thickening of the arterial wall in the trabeculae and follicles are also frequent (Nakagawa and Kajihara, 1979b).

4.4. Other Organs

Esophagogastric varices are universal, and mild to moderate venous congestion of mucosa of the gastrointestinal tract is common. Other organs do not show any significant change except in rare instances where marked obliterative changes in the superior mesenteric veins may lead to patchy ischemia of the intestine (N. C. Nayak, unpublished observations).

5. PATHOGENESIS AND ETIOLOGY

This aspect of tropical splenomegaly is at the same time the least understood and the most challenging. Searches for the cause and evolution of sustained portal hypertension in the absence of extrahepatic porto-splenic venous block or cirrhosis have caused concern and confusion.

5.1. Pathogenesis

It is true that although the liver is not cirrhotic, neither is it entirely normal. There is a variable degree of chiefly portal fibrosis which on occasion is severe

and extensive. In earlier reports it has been claimed that this fibrosis may be the cause of portal hypertension. This is, however, unlikely, since severity of the fibrosis and increased portal vein pressure are not necessarily related, and portal fibrosis alone as seen in a variety of other liver disorders may not be associated with significant portal hypertension (Leather, 1961; Siderys and Vellios, 1964; Mikkelsen *et al.*, 1965; Williams *et al.*, 1966; Banerji *et al.*, 1969). In schistosomiasis, where there is a "pipestem" type of fibrosis in the portal areas, the portal hypertension is attributed not to the fibrosis but to the occlusion of the venous radicles by the ova and by the resultant thrombophlebitis (Aufses *et al.*, 1959; Garcia-Palmieri and Marcial-Rajas, 1959).

Some early reports suggested that increased effluent from a large spleen may be the cause of portal hypertension (Tisdale *et al.*, 1959; Hallenback and Adson, 1961; Williams *et al.*, 1966; Siderys and Vellios, 1964). This also appears untenable, since even though the effective hepatic blood flow is increased in many patients with tropical splenomegaly, in others it is within normal range, and in a few it is even decreased. Moreover, increased splenic effluent seen in other conditions with massive splenomegaly are not associated with any significant rise in portal venous pressure.

Thickening of the medium and some of the smaller intrahepatic portal vein radicles and obliteration of the terminal branches seem to be consistent morphological features of tropical splenomegaly (Nayak and Ramalingaswami, 1969; Mikkelsen *et al.*, 1965; Aikat *et al.*, 1979; Okuda, 1979b). This change may also be specific to the disease, since it is not observed with any degree of frequency in either cirrhosis or other causes of severe portal hypertension without a block of the extrahepatic porto-splenic venous system. To be sure, the change is patchy and does not always involve every major branch of the portal vein. However, when autopsy material is very carefully sampled, these occlusive venous changes are always encountered.

Needle biopsy material unfortunately samples only the peripheral part of the liver, and, therefore, medium-sized veins do not appear in them. Radiological and hemodynamic data as well as observations on plastic casts and eroded material fully support the microscopic finding of oblitative and occlusive changes. The sustained high level of portal venous pressure is most certainly caused by some significant mechanical obstruction to the intrahepatic flow of portal venous blood, and pressure studies clearly indicate that the site of this obstruction is mostly at the presinusoidal level. Narrowing of segments of the intrahepatic portal vein branches and consequent rigidity of their normally extremely pliable wall could be consistent with these being the site of the increase resistance. The irregular and sometimes extensive portal fibrosis may well be the result of these vascular alterations, whereas dilatation of some of the other portal vein radicles possibly represents the effect of bypass of the obstruction through normally dilatable channels. In several of the livers from patients of tropical splenomegaly, mural thrombi and cavernomatous changes are seen in the major

intrahepatic portal vein branches (Mukherjee *et al.*, 1979; Futagawa *et al.*, 1980). It is, therefore, possible that the thickening of the walls of the medium-sized branches may also be a result of old organized mural thrombi. The inconspicuousness of the very small portal vein radicles and the development of multiple small channels may be effects of the process of occlusion and obliteration occurring in the more proximal vessels over a long period of time.

The exact sequence of events affecting the various levels of intrahepatic portal vein branches is uncertain. Some investigators believe that a phlebitic process initially affects the very small portal vein radicles which subsequently become obliterated and give rise to the portal hypertension which in turn leads to development of intimal thickening in the more proximal vessels as well as mural and luminal thrombi seen at various phases of evolution of the disease (Aikat *et al.*, 1979). On the other hand, Nayak and his colleagues (Nayak and Ramalingaswami, 1969; Mukherjee *et al.*, 1979) are of the opinion that the primary event is possibly the formation of mural thrombi in the medium sized and larger intrahepatic branches of portal vein which, in due course, are incorporated into the wall, imparting a marked thickening to the vessel, and alterations in the smaller radicles are the effect rather than the cause of this phenomenon. They have been able to demonstrate not only recanalized thrombi in the major portal vein branches in many cases but also have found frequent evidence of patchy involvement of the extrahepatic portal vein and its tributaries (Mukherjee *et al.*, 1979). Similar findings of thrombus formation in the vessels have also been demonstrated by Japanese workers (Okuda, 1979b).

Demonstration of collagen fiber deposition in the perisinusoidal space of the liver has created an interest in the possible role of this abnormality in the pathogenesis of portal hypertension in tropical splenomegaly (Tandon *et al.*, 1970; Sama *et al.*, 1971; Nataf *et al.*, 1979). It has been suggested that the beginning of portal hypertension in these patients can be ascribed to this sinusoidal abnormality which would give rise to hemodynamic evidence of presinusoidal hypertension. This view, however, needs confirmation, particularly with regard to the specificity of the morphological findings. It appears that the deposition of collagen in the perisinusoidal space of Disse is prominently observed not only in cirrhotic livers but also in a variety of other hepatic disorders. In experimental liver injury in animals, collagen deposition in the perisinusoidal space is frequently observed even when no evidence of portal hypertension is present. It is, therefore, difficult to ascribe an important role to this abnormality in the development and maintenance of portal hypertension in patients with tropical splenomegaly. Fiber formation may be result of vascular compromise since it is also seen in cases with extrahepatic porto-splenic venous block (N. C. Nayak, unpublished observation). Moreover, formation of collagen fibers in the perisinusoidal space would be expected to have a greater effect on diffusion of essential metabolites to and from the hepatocytes rather than to result in a

mechanical obstruction of sinusoidal blood flow. In tropical splenomegaly, abnormalities in liver functions are relatively infrequent. Also, accumulation of amyloid, another fibrillar material, even in very large amounts in the perisinusoidal spaces does not result in significant portal hypertension.

Hyperplastic nodules of hepatic parenchyma which are known to be an important cause of stretching of the hepatic and portal vascular bed in cirrhosis, thus leading to portal hypertension, are not frequent in livers of tropical splenomegaly patients even though a few of these nodules can sometimes attain relatively large sizes. When located in strategic positions close to the main branches of the portal vein, they possibly contribute in a small way to the resistance of portal venous flow (Sherlock *et al.*, 1966; Aikat *et al.*, 1979). On the other hand, it is unlikely that such nodules have any primary role in the genesis of portal hypertension, since even in more diffuse nodular hyperplasia affecting the entire liver significant portal hypertension is unknown.

5.2. Etiology

With the limitation in our knowledge on the pathogenesis of the essential lesions in tropical splenomegaly, views on the etiology have been almost totally speculative. Epidemiologic and clinical data, except for showing increased susceptibility in certain regions and countries, have not indicated any particular environmental factor to be strongly associated with the disease. The sex distribution appears to be variable, the disease affecting more males in India and more females in Japan. No particular dietetic or other habit seems to be more frequent in patients than in controls.

Because of the occurrence of the disease largely in the tropical and subtropical regions, several infections prevalent in these areas have been considered in the etiology of the disease. The African "big spleen disease" has in the past been ascribed to malarial infection. However, it appears that in countries of Africa, tropical splenomegaly and malarial splenomegaly are indeed two distinct entities. In the latter, the portal hypertension is not particularly prominent, epidemiologic, clinical, and immunologic evidence of malarial infection can be easily demonstrated, and the hepatic morphology is characterized by moderate to heavy infiltration of the sinusoids by lymphocytes and not by changes characteristic of tropical splenomegaly. In the Indian and other Asian cases of tropical splenomegaly, no evidence of malarial infection is seen. Also, during the period when malaria was almost eradicated in the former country, tropical splenomegaly continued to persist.

Schistosomiasis, which is endemic in some parts of Japan, is not seen in India except in very tiny pockets, and wherever tropical splenomegaly is common an association with schistosomal infection has not been observed. There has been some interest recently on the possible role of hepatitis B virus in the

eitology of tropical splenomegaly. Our own studies as well as those of others (Nayak *et al.*, 1977; Sakata *et al.*, 1979; Satoh *et al.*, 1980) on identifying markers of this infection in the liver and the serum, on the other hand, have not indicated that this agent may be causally responsible for tropical splenomegaly.

During the last decade considerable evidence has accumulated to show that continued ingestion of certain chemicals including arsenic and vinyl chloride may result in portal hypertension very similar to that seen in tropical splenomegaly (Morris *et al.*, 1974; Huet *et al.*, 1975; Thomas *et al.*, 1975; Smith *et al.*, 1976). Interestingly, the hepatic vascular changes in such individuals are identical or very similar to those seen in tropical splenomegaly. This obviously raises the question of whether in the latter patients arsenic or vinyl chloride intoxication operates to induce the hepatic alterations and portal hypertension. Data available to date fail to reveal such associations, and it remains to be seen as to whether some other chemical or biological toxin is responsible for the disease.

The syndrome of idiopathic portal hypertension chiefly showing peri-sinusoidal fibrosis has been reported in patients on long-term immunotherapy for renal transplantation or for other diseases (Nataf *et al.*, 1979). Not only has this type of fibrosis has been incriminated in the genesis of portal hypertension in these patients as well as in tropical splenomegaly (Tandon *et al.*, 1970) but also, by comparison, an immunologic basis for the development of tropical splenomegaly has been suggested. Although this is an attractive proposition by itself, there is as yet no direct or indirect evidence to incriminate an immunologic anomaly in initiating and maintaining this disease process. Attempts at determining HLA typing of such patients have not shown any significant difference between them and the control population (Takano *et al.*, 1979; Satoh *et al.*, 1980).

Thrombosis of the portal venous channels and tributaries, whether it is the cause, and effect, or a mere association of the disease, is undoubtedly a consistent and important finding. The causative factor for this vascular abnormality is unknown. Inflammation, infection, and toxic injury have all been incriminated from time to time but without any definitive evidence. It is possible that a malfunctioning of either the platelets or other coagulation factors in these patients makes them prone to development of thrombosis.

6. PROBLEMS OF MANAGEMENT

In a chronic, somewhat incapacitating disease with episodes of bleeding, the clinician is constantly faced with the problem of immediate and long-term management. As already stated, the disease seems to have a relatively benign course, and if the bleeding episodes are not associated with hepatic decompensation, minimal medical management is necessary to tide the patient over these

crises. Patients have been known to have several bleeds without any deterioration in the general health or any particular threat to life. Thus, although active surgical interference might not be indicated in most patients, in patients for whom immediate relief for bleeding may not be available, it is considered beneficial to perform a shunt surgery. In fact, Nundy and Tandon (1980) suggest that emergency lienorenal shunt is an effective and comparatively safe procedure which should be performed for bleeding esophageal varices in such patients. The decision to proceed with a surgical intervention, therefore, would depend on the type of presentation of the case, and if a patient has not bled or has bled insignificantly and infrequently, one may still manage the patient conservatively.

The other important manifestations of the disease are the hugely enlarged spleen and hematological deficiencies, both of which may necessitate consideration of a splenectomy. Many experienced investigators believe that in case a splenectomy is performed, a simultaneous lienorenal shunt should be carried out. With advancement in surgical techniques and in postoperative care of such cases, it would be possible to give many of these patients proper and timely surgical relief.

7. CONCLUSIONS AND FUTURE PROSPECTS

It is apparent that tropical splenomegaly or hepatoportal sclerosis is a distinct disease entity in its own right. It is also important to realize that this disease is frequent in many parts of the world, particularly in relatively overpopulated areas. The disease carries a high degree of morbidity and because of a prolonged course is amenable to being properly tackled once a firm diagnosis is made. Extensive work on the clinical, radiological, hemodynamic, and morphological aspects of this disease has now made it possible for the clinician to strongly suspect the condition and for the pathologist to confirm the diagnosis. Appropriate medical and surgical management can also go a long way in preventing suffering and considerably improving the state of life. What is, however, more discouraging is our almost total ignorance about the etiology and pathogenesis of the condition. Elucidation of these lacunae only can lead to a more satisfying management of the problem in terms of preventive and curative measures. There is little doubt now that at some time during the evolution of the disease venous thrombosis and sclerosis occur as key events and are responsible for certain important sequelae. The mechanisms of these changes are entirely unknown.

All future studies, therefore, must aim at clarifying the role of these venous changes in the pathogenesis of the disease and their prevention. Unfortunately, as yet, experimental models have not been set up to replicate the condition in the laboratory. Some attempts to induce thrombosis in the portal venous system of rats, rabbits, and monkeys have yielded disappointing results (N. C. Nayak,

unpublished data). The main difficulties in these models have been the quick resorption of the autologous thrombi and the technical difficulty in introducing multiple showers of thrombi in the animals. In case a suitable experiment can be designed to reproduce the important pathological changes seen in human patients, stepwise studies would help in the understanding of the evolution of the disease and also make it possible for further studies to be planned. Simultaneously, all efforts should be continued to collect by the use of currently available technology more information regarding various likely abnormalities in these patients.

REFERENCES

- Aida, Y., and Okudaira, M., 1979a, Pathological studies of the liver with idiopathic portal hypertension, in: *IPH Research Committee Report* (K. Okuda, ed.), Japan Ministry of Health and Welfare, Tokyo, pp. 10–22.
- Aida, Y., and Okudaira, M., 1979b, Pathological and histometrical studies of the liver with idiopathic portal hypertension, in: *IPH Research Committee Report* (K. Okuda, ed.), Japan Ministry of Health and Welfare, Tokyo, pp. 56–62.
- Aikat, B. K., Bhusnurmath, S. R., Chuttani, P. N., Mitra, S. K. and Dutta, D. V., 1979, The pathology of non-cirrhotic portal fibrosis, *Hum. Pathol.* 10:405–418.
- Aufses, A. H., Jr., Schaffner, F., Rosenthal, W. S., and Herman, B. G., 1959, Portal venous pressure in “pipestem” fibrosis of liver due to schistosomiasis, *Am. J. Med.* 27:807–810.
- Banerji, L., Sood, S. K., and Nayak, N. C., 1969, Spectrum of minor histological alterations in livers obtained from medicolegal autopsies, *Indian J. Pathol. Bacteriol.* 12:8–13.
- Banti, G., 1883, Dell’ anaemia splencia, *Arch. Scuola Anat. Patol. (Firenze)* 2:53.
- Basu, A. K., 1980, Non-cirrhotic portal fibrosis with portal hypertension, *Trop. Gastroenterol.* 1(3):117–123.
- Basu, A. K., and Aikat, B. K. (eds.), 1963, *Tropical Splenomegaly*, Butterworths, London.
- Basu, A. K., and Guharay, B. N. (eds.), 1978, *Noncirrhotic Portal Fibrosis with Portal Hypertension*, National Academy of Medical Sciences, India, New Delhi.
- Basu, A. K., Boyer, J., Bhattacharya, R., Basu Mallick, K. C., and Sen Gupta, K. P., 1967, Non-cirrhotic portal fibrosis with portal hypertension—a new syndrome, Part I, *Indian J. Med. Res.* 55:336–359.
- Basu Mallick, K. C., Sen Gupta, K. P., Basu, A. K., Biswas, S. K., Pal, N. C. and Boyer, J., 1967, Non-cirrhotic portal fibrosis with portal hypertension—a new syndrome, Part II, *Indian J. Med. Res.* 55:351–359.
- Benhamou, J. P., Gillemot, R., Triot, R., Leger, L., and Fauvert, R., 1962, Hypertension portale essentielle, *Presse Med.* 70:2397–2399.
- Bhalerao, R. A., Deodhar, K. P., and Nerurkar, G., 1968, Primary portal hypertension, *J. Postgrad. Med.* 14(3):129–132.
- Boyer, J. L., Sengupta, K. P., Biswas, S. K., Pal, N. C., Basu Mallick, K. C., Iber, F. L., and Basu, A. K., 1967, Idiopathic portal hypertension comparison with portal hypertension of cirrhosis and extrahepatic portal vein obstruction, *Ann. Intern. Med.* 66:41–68.
- Boyer, J. L., Hales, M. R., and Klatekin, G., 1974, “Idiopathic” portal hypertension due to occlusion of intrahepatic portal veins by organized thrombi, *Medicine (Baltimore)* 53:77–91.
- Cook, J., McFadzean, A. J. S., and Todd, S., 1963, Splenectomy in cryptogenetic splenomegaly, *Br. Med. J.* 2:337–344.

- De, M. N., 1932, Some facts about the incidence of splenomegaly in Bengal, *Indian J. Med. Res.* 19:1929.
- De, M. N., and Tribedi, B. P., 1939, The pathogenesis of common types of splenomegaly met with in India, *Indian Med. Gaz.* 74:9–15.
- Futagawa, S., Fukagawa, M., Horisawa, M., Musha, H., Ito, T., Sugiura, M., Kameda, H., and Okuda, K., 1980, Portographic liver changes in idiopathic non-cirrhotic portal hypertension, *Am. J. Poentgenol.* 134:917–923.
- Garcia-Palmieri, M. R., and Marical-Rajas, R. A., 1959, Portal hypertension due to schistosomiasis mansoni. A clinico-pathological correlation, *Am. J. Med.* 27:811–816.
- Hallenback, G. A., and Adsen, M. A. 1961, Esophagogastric varices without hepatic cirrhosis: A clinical study of treatment for their bleeding in 72 cases, *Arch. Surg.* 83:370–383.
- Hamilton, P. J. S., Richmond, J., Donaldson, G. W. K., Williams, R., Hutt, M. S. R., and Lugumba, V., 1967, Big spleen disease, *Br. Med. J.* 3:823–825.
- Huet, P. M., Guillaume, E., Cote, J., Legare, A., Lavoie, P., and Viallet, A., 1975, Non-cirrhotic presinusoidal portal hypertension associated with chronic arsenical intoxication, *Gastroenterology* 68:1270–1277.
- Iber, F. L., 1969, Obliterative portal venopathy of the liver and “idiopathic portal hypertension,” *Ann. Intern. Med.* 71:660–661.
- Iber, F. L., 1970, Portal hypertension in the presence of normal liver morphology, *Ann. N.Y. Acad. Sci.* 170:115–126.
- Imanaga, H., Yamamoto, S., and Kunayanagi, Y., 1962, Surgical treatment of portal hypertension according to state of intrahepatic circulation, *Ann. Surg.* 155:42–50.
- Indian Council of Medical Research, 1969, *Workshop on Noncirrhotic Portal Fibrosis*, Indian Council of Medical Research, New Delhi.
- Kage, M., Koga, M., Arakawa, M., Kojiro, M., Kawasaki, H., Nakaehima, T., Inokuchi, K., Kabayashi, M., Beppu, K., and Sakata, H., 1979a, The histopathology of IPH, in: *IPH Research Committee Report* (K. Okuda, ed.), Japan Ministry of Health and Welfare, Tokyo, pp. 33–43.
- Kage, M., Koga, M., Arakawa, M., Kojiro, M., Kawasaki, H., and Nakashima, T., 1979b, Histological comparison of idiopathic portal hypertension in Japan and non-cirrhotic portal fibrosis in India, in: *IPH Research Committee Report* (K. Okuda, ed.), Japan Ministry of Health and Welfare, Tokyo, pp. 44–55.
- Kobayashi, Y., Inokuchi, K., Saku, M., Beppu, K., Sakake, H., and Kohno, H., 1976, Epidemiology of idiopathic portal hypertension based on a nationwide survey, in: *1976 Report of the Ministry of Health and Welfare Research Committee on Idiopathic Portal Hypertension*, M. (Suguiara, ed.), Japan Ministry of Health and Welfare, Tokyo, pp. 10–15.
- Koga, M., Kage, M., Arakawa, M., Kojiro, M., Kawasaki, H., and Nakashima, T., 1979, Pathological study of IPH, in: *IPH Research Committee Report* (K. Okuda, ed.), Japan Ministry of Health and Welfare, Tokyo, pp. 23–32.
- Leather, H. M., 1961, Portal hypertension and gross splenomegaly in Uganda, *Br. Med. J.* 1:15–18.
- Leevy, C. M., Popper, H., and Sherlock, S., 1977, Diseases of the liver and biliary tract: Standardization of nomenclature, diagnostic criteria and diagnostic methodology, in: *Fogarty International Center Proceedings No. 22* (C. M. Leevy, H. Popper, and S. Sherlock, eds.), United States Government Printing Office, Washington, p. 16.
- Marleau, D., Villeneuve, J. P., Huet, P. M., Cote, J., Lafortune, M., Legare, A., Lavoie, P., and Viallet, A., 1975, Non-cirrhotic portal hypertension (IPH): Radiological and haemodynamic evaluation of cases by combined hepatic and umbilicportal catheterization, *Gastroenterology* 67:813.
- Marsden, P. D., Hutt, M. S. R., Wilks, N. E., Veller, A., Blackman, V., Shah, K. K., Connor, D. H., Hamilton, P. J. S., Banwell, J. G., and Lunn, H. F., 1965, An investigation of tropical splenomegaly at Mulago Hospital, Kampala, Uganda, *Br. Med. J.* 1:89–92.

- McMichael, J., 1934, The pathology of hepatolienal fibrosis, *J. Pathol. Bacteriol.* 39:481–502.
- Mikkelsen, W. P., Edmondson, H. A., Peters, R. L., Redeker, A. G., and Raynolds, T. B., 1965, Extra and intrahepatic portal hypertension without cirrhosis (hepato-portal sclerosis), *Ann. Surg.* 162:602–620.
- Miller, M. C., and Brandt, J. L., 1962, Portal hypertension in the absence of hook liver disease and vascular obstruction, *Am. J. Dig. Dis.* 37:442–448.
- Morris, J. S., Schmid, M., Newman, S., Scheuer, P. J., and Sherlock, S., 1974, Arsenic and non-cirrhotic portal hypertension, *Gastroenterology* 66:86–94.
- Mukherjee, A. K., Ramalingaswami, V., and Nayak, N. C., 1979, Hepatoportal sclerosis—its relationship to intrahepatic portal venous thrombosis, *Indian J. Med. Res.* 69:152–160.
- Nakagawa, S., and Kajihara, Y., 1979a, Pathology of the spleen in idiopathic portal hypertension (1st. report) in: *IPH Research Committee Report* (K. Okuda, ed.), Japan Ministry of Health and Welfare, Tokyo, pp. 124–130.
- Nakagawa, S., and Kajihara, Y., 1979b, Pathology of the spleen in idiopathic portal hypertension—lesion of the arterial system, in: *IPH Research Committee Report* (K. Okuda, ed.), Japan Ministry of Health and Welfare, Tokyo, pp. 131–138.
- Nataf, C., Feldmann, G., Lebre, D., Degott, C., Descamps, J. M., Rueff, B., and Benhamou, J. P., 1979, Idiopathic portal hypertension (perisinusoidal fibrosis) after renal transplantation, *Gut* 20:531–537.
- Nayak, N. C., and Ramalingaswami, V., 1969, Obliterative portal venopathy of the liver, *Arch. Pathol.* 87:359–369.
- Nayak, N. C., Dhar, A., Sachdeva, R., Mittal, A., Seth, H. N., Sundarsanam, D., Reddy, B., Waghlikar, U. L., and Reddy, C. R. R. M., 1977, Association of human hepatocellular carcinoma and cirrhosis with hepatitis B virus surface and core antigen in the liver, *Int. J. Cancer* 20:643–654.
- Nundy, S., and Tandon, B. N., 1980, Emergency operations for bleeding oesophageal varices—a case for doing splenectomy and lienorenal shunts, *Trop. Gastroenterol.* 1(10):27–31.
- Okuda, K., 1971a, Non-cirrhotic portal fibrosis (NCPF) in India, in: *IPH Research Committee Report* (K. Okuda, ed.), Japan Ministry of Health and Welfare, Tokyo, pp. 44–46.
- Okuda, K. (ed.), 1979b, *IPH Research Committee Report*, Japan Ministry of Health and Welfare, Tokyo.
- Osler, W., 1900, On splenic anaemia, *Am. J. Med. Sci.* 119:54.
- Polish, E., Christie, J., Cohen, A., and Sullwan, B., Jr., 1962, Idiopathic presinusoidal portal hypertension (Bantis syndrome), *Ann. Intern. Med.* 56:624–627.
- Sakata, H., Inokuchi, K., Saku, M., Beppu, K., Kohno, H., and Kabayashi, M. 1979, Epidemiologic and clinical investigation in patients with idiopathic portal hypertension in patients with idiopathic portal hypertension in Japan, in: *IPH Research Committee Report* (K. Okuda, ed.), Japan Ministry of Health and Welfare, Tokyo, pp. 166–169.
- Sama, S. K., Bhargava, S., Gopinath, N., Talwar, J. R., Nayak, N. C., Tandon, B. N., and Wig, K. L., 1971, Non-cirrhotic portal fibrosis, *Am. J. Med.* 51:160–169.
- Sato, H., Iwasaki, Y., Ohkubo, A., Beppu, T., Futagawa, S., Sugiura, M., and Juji, T., 1980, Human leukocyte antigen (HLA) in patients with idiopathic portal hypertension (IPH), *Tohoku J. Exp. Med.* 131:393–397.
- Sherlock, S., Feldman, C. A., Moran, B., and Scheuer, P. J., 1966, Partial nodular transformation of the liver with portal hypertension, *Am. J. Med.* 40:195–203.
- Sideris, H., and Vellios, F., 1964, Portal hypertension without cirrhosis or extrahepatic obstruction. Report of a case, *Am. J. Surg.* 108:785–789.
- Smith, P. M., Crossley, I. R., and Williams, D. M. J., 1976, Portal hypertension in vinyl-chloride production workers, *Lancet* 2:602–604.
- Sugiura, M., and Ichihara, S., 1971, Etiology of idiopathic portal hypertension (hepatic fibrosis), *Jpn. J. Gastroenterol.* 68:434–436.

- Takano, Y., Yoshida, K., Tsukada, K., and Muto, T., 1979, Frequencies of HL-A antigens in idiopathic portal hypertension, in: *IPH Research Committee Report* (K. Okuda, ed.), Japan Ministry of Health and Welfare, Tokyo, pp. 116–117.
- Tandon, B. N., Lakshminarayan, R., Bhargava, S., Nayak, N. C., and Sama, S. K., 1970, Ultrastructure of the liver in noncirrhotic portal fibrosis with portal hypertension, *Gut* 11:905–910.
- Thomas, L. B., Popper, H., Berk, P. D., Selikoff, I., and Falk, H., 1975, Vinyl-chloride-induced liver disease from idiopathic portal hypertension (Bantis syndrome) to angiosarcomas, *N. Engl. J. Med.* 292:17–22.
- Tisdale, W. A., Klatskin, G., and Glen, W. W. L., 1959, Portal hypertension and bleeding oesophageal varices: Their occurrence in the absence of both intrahepatic and extrahepatic obstruction of the portal vein, *N. Engl. J. Med.* 26:209–218.
- Williams, R., Parsanion, A., Somers, K., and Hamilton, P. J. S., 1966, Portal hypertension in idiopathic tropical splenomegaly, *Lancet* 1:329–333.

Immune Complex Nephropathy in the Tropics

JACOB L. NGU AND JOHN F. SOOTHILL

1. INTRODUCTION

There have, in the past 20 years, been a number of reports and reviews on immune complex (IC) nephropathy associated with various parasitic infections in the tropics. Although these may be important, nephritis in tropical countries is not exclusively caused by a parasitic etiology. The range of etiologic factors in IC nephropathy established in temperate regions also obtains in the tropics in addition to parasites. Admissions data indicate that the frequency of IC nephropathy is over ten times greater in tropical than in temperate regions (Kibukamusoke, 1966; Ngu, 1969). It has been estimated that in some parts of Africa the hospital admission rate of patients with the nephrotic syndrome is 100 times higher than that in the United States (Lancet, 1980). This high prevalence in the tropics can be accounted for, at least in part, by the range of common parasite-induced IC diseases superimposed on the other antigen-antibody (Ab-Ag) systems known to cause renal damage elsewhere.

2. COMPOSITION AND NATURE OF IMMUNE COMPLEXES

An IC is formed when antibody interacts with its cognate antigen. This occurs all the time as a component of the normal immune response. These

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complexes do not usually cause disease as they are rapidly cleared from the circulation by cells of the reticuloendothelial system (RES) (Weigle, 1961), in particular the sinusoidal mononuclear cells of the liver (Mannik and Arend, 1971). Only occasionally does tissue injury accrue from IC formed *in vivo*. The nature of complexes formed depends on many factors such as the nature of the antigen and the number and density of its antigenic determinants, the Ab–Ag ratio and their concentration, the class and complement-fixing properties of the antibody, and the affinity of the antibody, that is, the strength of bond at an individual antigen binding site which results from the summation of attractive and repulsive forces (Steward, 1977).

The avidity or strength of polyvalent binding between the molecules also probably exerts considerable influence on the nature of complexes formed and their biological properties. A low-affinity antibody response is associated with defective antigen clearance (Alpers *et al.*, 1972), and such antibody fixes complement poorly when it reacts with antigen. Complement fixation greatly increases opsonization, so antibody classes and subclasses that activate complement through the efficient classical pathway (IgM and IgG) will eliminate antigen quickly. Immunoglobulin A probably and some antigens activate complement through the alternative pathway, which is less efficient. Nonetheless, IgA achieves effective antigen elimination (Stokes *et al.*, 1980). Immune complexes with low-affinity antibodies are not efficiently cleared from the circulation by the mononuclear phagocytic system, so the antigen they contain remains to be potentially pathogenic, in contrast to that in IC with high-avidity antibodies which are effectively cleared. The affinity of antibody matures with time during the course of the antibody response, but it also depends on a number of other factors, including the nature of the antigenic stimulus (Soothill and Steward, 1971), genetic factors (Soothill and Steward, 1971; Petty *et al.*, 1972), and macrophage (Morgan and Soothill, 1975) and lymphocyte function (Steward, 1977). Environmental factors also influence both antibody affinity and macrophage function; the effect of malnutrition is perhaps particularly important in the tropics (Passwell *et al.*, 1974; Coovadia and Soothill, 1976).

3. RENAL DAMAGE BY IMMUNE COMPLEXES

3.1. Mechanisms of Induction of Renal Damage by Immune Complexes

Glomerulonephritis occurs as a result of activation of complement in the glomeruli. This may happen when anti-glomerular basement membrane (GBM) antibody reacts with it, but this is not usually included in “immune complex nephropathy.”

Immune complex nephropathy occurs both when Ab–Ag complexes are formed in the circulation and are trapped in the glomerular filtering structures (or other renal tissue) or when antigens of nonrenal origin are lodged in the kidney so that an Arthus-reaction-type phenomenon occurs. The latter happens in at least one form of tubulointerstitial nephritis (Unanue *et al.*, 1967) and possibly in some cases of nephropathies associated with helminthic infections (see below).

Immune complexes cause damage by activating plasma mediator systems, particularly complement (C), or by reacting with cells that have receptors for Fc pieces of complexed immunoglobulin (Ig) or C3b or C3d. In most forms of IC nephropathy in man, C deposition occurs in the glomeruli and probably represents the major mediator of immunologic injury (Verroust *et al.*, 1974; Lambert *et al.*, 1974; Leber and McCluskey, 1974; Hunsicker, 1974; Fearon *et al.*, 1974; Berger *et al.*, 1974). The quantitatively and functionally predominant molecule of the complement system is C3. It may be activated by either the classical (antibody-dependent) or alternative (properdin) pathway (Figure 1). Immune complexes usually activate the classical pathway in which C1 (itself a complex—C1q, r, and s) activates C4 and C2 sequentially by enzymic cleavage; this leads to C3 cleavage, releasing a circulating small fragment, C3a, and large C3b which adheres to neighboring plasma membranes. C3b also activates a feedback loop for activation of more C3 which probably turns over slowly at all times. Besides the classical pathway acceleration, this feedback activity may be increased directly by certain alternative pathway activators which operate without antibody (it is possible that complexed IgA can activate complement in this way). Both pathways lead to the production of C5 convertase and activation of the late complement components.

During the C cascade reaction, fragments and cleaved products of C components are produced with biological properties that are important in the production of the lesions seen. The trimolecular complex C567 and the fragments C3a and C5a induce chemotaxis of polymorphonuclear leucocytes (PMN) which accumulate at sites of antibody–antigen–C localization (Bokisch *et al.*, 1969; Ward, 1974). C3a and C5a mediate anaphylatoxin activity (Vallota and Müller-Eberhard, 1973; Bokisch *et al.*, 1969). C3b promotes opsonization and immune adherence whereby PMN, platelets, and macrophages attach to the membrane-bound C component (Nishioka and Linscott, 1963; Linscott and Nishioka, 1963; Müller-Eberhard, 1968; Henson, 1969). After immune adherence through C3b receptors, the PMN release from their granules injurious proteolytic enzymes, basic peptides, etc. The process probably accounts for most of the damage in acute immunologic nephritis in which PMN participate (World Health Organization, 1977). The late sequence of events in C activation leads to lysis of target cells and bystander cells through the terminal C components.

Other plasma components that may be activated by IC and mediate immunologic damage include the kinin, coagulation, and fibrinolytic systems in all of

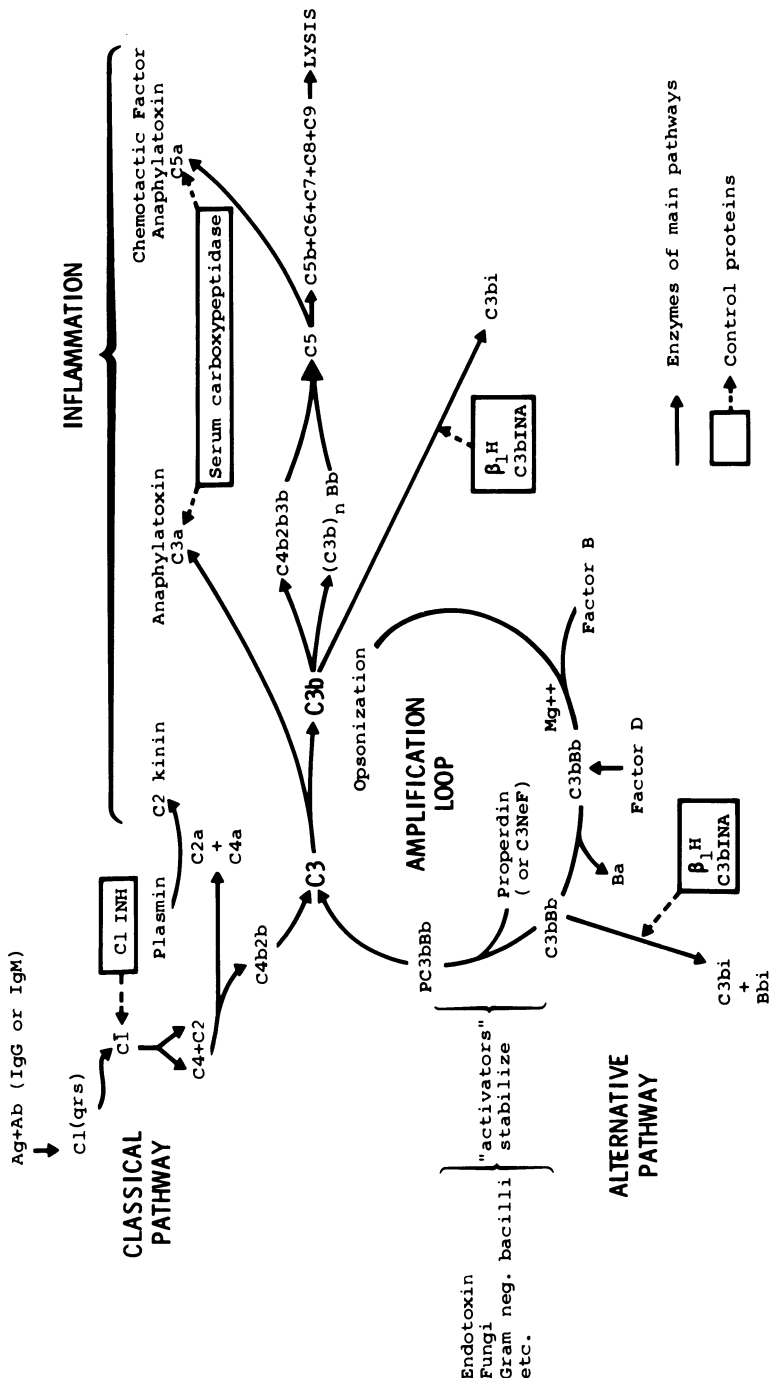


Figure 1. A simplified diagram of the complement system. The main reaction is the activation of C3, which probably occurs continuously at a low level, as a result of the amplification loop. The system may be activated by either the classical or the alternative pathway and is controlled by inhibitors (indicated by dashed arrows). In general, the system is a cascade of proteolytic enzymes, each activating the next. Activation of C3 (and C5) leads to release of mediator fragments which result in opsonization and inflammation; it also activates the lytic pathway (From Turner, 1982, reproduced by kind permission).

which the Hageman factor is involved. Hageman factor can bind to vascular membranes of mucopolysaccharide and collagen (Cochrane *et al.*, 1972), initiating coagulation by activating factor IX. The fibrin formed has chemotactic properties and also enhances vascular permeability (Marder, 1971). The Hageman factor also converts prekallikrein to kallikrein, resulting in chemotaxis of PMN and bradykinin production (Kaplan and Austen, 1970), and is involved in plasmin formation and fibrinolysis through activation of plasminogen proactivator (Austen, 1974). The activation of these systems may in turn involve the C cascade events—for example, plasmin may activate C1 (Ratnoff and Naff, 1967). The aggregation of platelets, deposition of fibrin, and coagulation in the glomeruli may cause damage through chemotactic, phlogistic, and other properties (Vassali and McCluskey, 1971; McIntosh *et al.*, 1971; Kincaid-Smith, 1972). Indeed fibrin which accumulates in Bowman's capsule promotes epithelial cell proliferation (Vassali and McCluskey, 1971; Morita *et al.*, 1973; Min *et al.*, 1974). The adherence of IC to other cells including platelets, PMN, monocytes, lymphocytes, and K cells (World Health Organization, 1977), may activate them causing damaging release of granular contents, phagocytosis, and nonphagocytic killing. These contribute to the glomerular damage.

3.2. Factors Influencing Localization and Pathogenicity of Immune Complexes and Chronicity of Renal Lesions

The size and nature of the complexes influence their rate of clearance by the RES and so their pathogenetic role. Complexes greater than 19×10^{-13} S and those that are C fixing are more readily cleared than smaller or non-C-fixing complexes which stay in the circulation, are ineffectively removed by the spleen, and may become fixed to the renal glomeruli. Such complexes may be formed by low-affinity antibodies or nonprecipitating antibody in antigen excess (Weigle, 1961; World Health Organization, 1977). Once they reach the glomeruli, the phagocytic capacity of mesangium affects their pathogenetic potential. The larger complexes preferentially accumulate in the mesangium, where they may not be particularly phlogogenic (Germuth and Rodriguez, 1973), whereas smaller complexes are trapped by the glomerular capillary basement membrane. When the mesangial function is depressed, for example, by drugs, or is overwhelmed, the pathogenetic potential of these IC is enhanced. The property of the antigen itself is also important. DNA binds to the collagen of the GBM, so IC with DNA as antigens are more likely to localize there than IC devoid of DNA (Izui *et al.*, 1976). The C3b receptors on the membrane of glomerular epithelial cells favor deposition of complement-containing complexes (Gelfand *et al.*, 1975). Hypertension enhances glomerular deposition of IC (Fisher and Bark, 1961; Wilens and Henderson, 1965), and decreased glomerular filtration impairs it (Germuth *et al.*, 1967).

Experiments suggest that all individuals are susceptible to acute glomerulonephritis when exposed to intravenous injection of large quantities of an antigen (acute serum sickness) but that the process recovers when the antigen is cleared. The problem remains why only some individuals are liable to chronic glomerulonephritis following repeated injections (Dixon *et al.*, 1961; Germuth and Rodriguez, 1973) and whether similar factors operate in human chronic soluble-complex glomerulonephritis. One factor is the genetic (and probably environmental) variation of affinity of antibody affinity and macrophage clearance function, mentioned above, which is related to such susceptibility (Devey and Steward, 1980). Animals susceptible to chronic IC disease are recognizable by their antibody response (Dixon *et al.*, 1961) and by their making complexes of an intermediate size (Germuth and Rodriguez, 1973).

If continual deposition of such complex were the mechanism, the continual availability of antigen would be required, but in most IC nephropathy in the tropics associated with parasitic infection, elimination of the parasite does not induce remission (see below). Alternative mechanisms would therefore be expected to operate.

The initial IC-induced glomerulonephritis (GN) may damage the GBM which, in turn, may cause further damage mediated by C activated through the alternative pathway [GBM fragments produced by pronase and glycoprotein constituents of GBM activate the alternative pathway of C (Lambert *et al.*, 1973, 1974)]. Foreign substance without antibody may cause damage locally by activating C through the alternative pathway, for example, pneumococcal GN (Hyman *et al.*, 1975) and perhaps the membranoproliferative GN which follows β -hemolytic streptococcal infection (Jenis *et al.*, 1974). Other possible mechanisms for chronicity include autoantibodies to antigens released from damaged glomeruli, antiglobulin antibodies, immunoconglutinins, etc., IC deposits in the glomeruli, cross reactivity of parasitic antigens with host antigens, and continued deposition of complex containing other antigens in patients whose clearance mechanism is saturated.

4. DIAGNOSIS OF IMMUNE COMPLEX NEPHROPATHY

Immune complex nephropathy is diagnosed by the demonstration of deposits of immunoglobulin (Ig) and, usually, C deposits in the glomeruli. This is usually done using immunofluorescence tests. Sometimes the antigen in the deposits and the specificity of the Ig in the kidney can be demonstrated after elution.

The pattern of deposition and Ig class/subclass on immunofluorescence is sometimes of prognostic value in certain forms of IC nephropathy (see Section

5.1.1a on malaria). The deposits may be intense or sparse and generalized or involving only segments of GBM or mesangium. Granules may be coarse and unevenly distributed (as in diffuse proliferative GN) or fine, uniformly distributed along GBM, giving a smoother pattern. Such appearances must be distinguished from the linear deposition of anti-GBM. In advanced disease, the complexes may be detectable only in the least damaged glomeruli. Immunoglobulin G is the most frequent Ig class seen, but more than one Ig class may be found together. Complement components and occasionally antigen may also be detected in the glomeruli by immunofluorescence. Usually C3 and components of both classical (e.g., C4) and alternative (e.g., properdin) pathways are detected, since classical pathway activation by IgG or IgM antibody activates the feedback that involves the alternative pathway (Figure 1). However, occasionally only C3 and the alternative pathway components are found, as in IgA nephropathy.

Evidence in the blood of activation of complement, such as circulating split products (Soothill and Hendrickse, 1967) or the presence of immunoconglutinin, an autoantibody to activated C3 (Ngu and Blackett, 1970), and detection of circulating IC (World Health Organization, 1977) provide some support for the diagnosis of IC nephropathy, but these phenomena occur in infections generally and in many other diseases. There are many tests that detect complexes; they depend on a number of characteristics (e.g., size, class, complement-fixing properties, presence of complement components), so they do not correlate precisely. More precise information can be obtained by better characterization of the complexes. Thus, although all patients with SLE have heterogenous circulating IgG complexes, those with nephritis have IC of intermediate size similar to the complexes described by Germuth in experimental glomerulonephritis (Levinsky *et al.*, 1977), and although most patients with Henoch–Schonlein purpura have circulating IgA complexes, those with nephritis have IgG complexes too (Levinsky and Barratt, 1979). Such discriminatory techniques are, regrettably, not yet generally available for diagnostic purposes.

5. ETIOLOGIC FACTORS

Increasing numbers of Ag–Ab systems of parasitic and nonparasitic origin are being incriminated in IC nephropathy seen in the tropics (Table I). The antigens may be exogenous and endogenous (e.g., in SLE) and include diseases observed throughout the world with the addition of other infections of local significance (Table II). The antigen was not identified in the majority of the cases cited in Table I.

Table I
Immune Complex Nephropathy in Nigerian Adults and in
Cameroonian Adults and Children^a

Etiology	Lagos 1966–1968 (Number of cases)	Yaoundé 1974–1981 (Number of cases)
Undetermined	28 ^b	57 ^c
<i>Plasmodium malariae</i>	—	3
Poststreptococcal	2	17
Leprosy	—	3
<i>Loa loa</i>	—	5
<i>Onchocerca volvulus</i>	—	19
Hepatitis B virus	—	5
Systemic lupus erythematosus	1	4
Goodpasture syndrome	—	1
Berger's disease (IgA nephropathy)	—	2
Henoch–Schönlein purpura	—	2
Wegener's granulomatosis	—	1

^aJ. L. Ngu, unpublished data.

^b*P. falciparum* parasitemia was consistently found in 18 of these.

^c*P. falciparum* parasitemia was found at various times in 16 of these.

Table II
Etiologic Factors in Immune Complex Nephropathies
in the Tropics

Exogenous Antigens
Parasitic
Malarial: <i>Plasmodium malariae</i> , <i>P. falciparum</i>
Schistosomal: <i>Schistosoma mansoni</i> , <i>S. haematobium</i>
Filarial: <i>Loa loa</i> , <i>Onchocerca volvulus</i>
Others: Trypanosomes, <i>Toxoplasma gondii</i>
Viral
Hepatitis B, Epstein–Barr
Bacterial
Nephritogenic streptococci, enterococcus, leprosy, <i>Treponema pallidum</i>
Iatrogenic
Drugs, chemicals
Endogenous Antigens
Nuclear
Systemic lupus erythematosus
Renal tubule brush border antigens
Sickle cell anemia
Tumor

5.1. Exogenous Antigens

5.1.1. Parasitic

Many parasites release soluble antigens into the circulation which evoke antibody responses. These antigens may differ at the different stages of the parasite. They may be excretory and secretory products as well as somatic ones. Some parasites have a remarkable capacity for antigenic variation, especially *Trypanosoma*, and some (e.g., *Schistosoma*) incorporate host antigens on their surface. Parasitic infection may cause alteration of components of host cells, which may become antigenic.

It is not surprising, therefore, that IC derived from these are extremely complex. A common denominator, however, of all parasite-associated IC nephropathies is that the infections are chronic and provide a continued source of antigens. The best-studied example is Quartan malaria nephropathy (QMN).

5.1.1a. Malarial Immune Complex Nephropathy

This is one of the best-studied Ag–Ab systems associated with renal damage. It occurs in areas endemic for malaria and is characterized by proteinuria and a variety of histopathological lesions, particularly in the glomeruli. Following acute *P. falciparum* malaria, antigens are released into the circulation (McGregor *et al.*, 1968; Wilson *et al.*, 1969; Wilson *et al.*, 1975 a, b; Wilson, 1970; Williams and Houba, 1972; Houba and Williams, 1972). However, circulating *P. malariae* antigens are more difficult to detect (Houba *et al.*, 1976 a, b).

There is some evidence of complement activation in malaria in man and other animals. Decreases in C1, C2, and C3 serum levels have been found with *P. knowlesi* parasitemia in rhesus monkeys. However, in *P. falciparum*- or *P. brasilianum*-infected owl monkeys, an initial increase in certain C components (C4, C3, and properdin factor B) is followed by a fall which may last several months (Lambert and Houba, 1974).

These antigens form IC detectable in sera, and the complexes are deposited in the glomeruli of patients (Houba *et al.*, 1976b). The complexes have been eluted from kidney biopsies, the antigen identified, and the plasmodial antibodies recovered from the eluates (Allison *et al.*, 1969; Houba *et al.*, 1976b; Bhamarapravati *et al.*, 1973).

i. Types of Immune Complex Glomerulonephritis of Malarial Origin. There are two types of IC nephropathy induced with malarial infection in man.

The Acute (Transient) Reversible Form. This occurs shortly after an acute episode of *P. falciparum* fever in heavily parasitized patients. They have pro-

teinuria of varying severity, and nephrotic syndrome has been observed in some cases (Berger *et al.*, 1967; Allison *et al.*, 1969). There is a range of renal histopathological changes including mesangial proliferation with hyperplasia and hypertrophy of endothelial cells and sometimes glomerular hypercellularity (Spitz, 1946; Boonpucknavig and Sitprija, 1979). Deposits containing IgM, IgG, and C3 occur in the glomerular mesangium and along the basement membrane, and *P. falciparum* antigen may be detected in the glomeruli (Bhamarapravati *et al.*, 1973; Boonpucknavig and Sitprija, 1979; Houba, 1979). Electron-dense deposits are seen in the subendothelial and paramesangial areas (Boonpucknavig and Sitprija, 1979), compatible with an IC lesion (Houba, 1979). During the acute phase of the disease, a decrease in serum C3 and C4, fibrin degradation products, and circulating IC may be detected (Neva *et al.*, 1974; Bhamarapravati *et al.*, 1973; Futrakul *et al.*, 1974; Srichaikul *et al.*, 1975; Ree, 1976).

Resolution of light and electron microscopy and immunofluorescence abnormalities is seen within 4–6 weeks after curative antimalarial treatment (Boonpucknavig and Sitprija, 1979). The picture resembles acute serum sickness glomerulonephritis.

Chronic (Progressive) Form. This is particularly associated with *P. malariae* infection, although we do not know why. It causes the nephrotic syndrome followed by chronic renal failure and hypertension. It may occur at any age, but particularly in children between the ages of 3 and 8 years, with a peak incidence at 5 years (Gilles and Hendrickse, 1960; Hendrickse and Adeniyi, 1979). The high incidence reported in Ugandan adults (Kibukamusoke, 1966; Kibukamusoke *et al.*, 1967) is not seen in Nigerian adults (Ngu and Monekosso, 1968, Ngu, 1969).

The disease characteristically starts with periorbital edema, particularly on waking up, which later becomes generalized. Most patients have fever at the onset, which in a few may be Quartan in periodicity (Hendrickse and Adeniyi, 1979). There is marked ascites, and half have hepatosplenomegaly (Hendrickse and Adeniyi, 1979). The blood pressure is usually normal in the early stages of the disease, in contrast to nephrotic syndrome of poststreptococcal types in which hypertension is usual. The proteinuria in QMN is poorly selective on differential protein clearance studies in most cases (Soothill and Hendrickse, 1967; Adeniyi *et al.*, 1976, 1979). Some patients die early of intercurrent infection, etc., but most progress to chronic renal failure within 5 years (Hendrickse and Adeniyi, 1979) despite all treatment; a few recover (Adeniyi *et al.*, 1976). It is not certain whether antimalarial or cyclophosphamide treatment contributes to this, but azathiaprime and probably steroids make some worse. Early descriptions of the renal histological picture in QMN were conflicting and misleading, as some of the cases were probably of poststreptococcal origin (Ngu, 1969).

However, since then, a characteristic form of glomerular pathology—Quartan malaria nephropathy (QMN)—has been recognized. The main findings are

thickening of GBM of the capillaries, particularly the subendothelial part, giving rise to "double contour of plexiform arrangement of PAS-positive argyrophilic fibrils" (Hendrickse *et al.*, 1972; Hendrickse and Adeniyi, 1979). This occurs segmentally in only a few glomeruli in the early stages but extends to involve more glomeruli, leading to progressive narrowing and obliteration of the capillary lumen, and in the advanced form there is total glomerular sclerosis. "Cellular proliferation is typically absent, although occasional foci of hypercellularity may be seen" (Hendrickse and Adeniyi, 1979). Crescents are unusual. Tubular atrophy and interstitial cellular infiltration occur. The electron-dense subepithelial deposits characteristic of poststreptococcal mesangial proliferative nephritis are not found (Hendrickse *et al.*, 1972; Morel-Maroger *et al.*, 1975).

Immunofluorescence studies show IC deposits of coarse to fine granular patterns (Ward and Kibukamusoke, 1969; Allison *et al.*, 1969; Houba *et al.*, 1970, 1971, 1976b; Hendrickse *et al.*, 1972). Both IgG and IgM were detected in 96% of biopsies, C3 in 66%, and *P. malariae* antigen in 25%. Most eluates from renal biopsies contained anti-*P. malariae* antibody (Houba *et al.*, 1976b).

Attempts have been made to correlate IgG subclasses in immune deposits and patterns of immunofluorescence with prognosis (Houba and Lambert, 1974). Most of those who improved have coarse granular deposits with IgG3 subclass, either alone or combined with others, but never IgG2. In contrast, cases showing fine granular (diffuse) immunofluorescence with IgG2 are usually nonresponders and have a poor prognosis. There is, however, no apparent relationship between histological grading on light microscopy and the immunofluorescence findings (Hendrickse *et al.*, 1972).

Decreased serum C has also been reported in children (Soothill and Hendrickse, 1967) and adults (Ngu and Monekosso, 1968; Ngu and Blackett, 1970) with the nephrotic syndrome and malaria parasitemia, but normal serum C levels in patients with chronic Quartan malaria infection have been reported by others (Cameron and Kibukamosoke, 1971; Lambert and Houba, 1974), perhaps because compensating increased synthesis of C varies. This explanation is in keeping with the finding of increased turnover of radiolabeled C1q during peaks of *P. falciparum* parasitemia (Srichaikul *et al.*, 1975) and the detection of split products of C3 and macromolecular forms of C3 in sera of nephrotic children with Quartan malaria (Soothill and Hendrickse, 1967).

ii. Mechanism of Perpetuation of Chronic Lesions. Constant availability of antigen in QMN would understandably result in continual IC formation. This is unlikely to account for the persistence of lesions, as intensive antimalarial therapy does not cause remission. Furthermore, detection of *P. malariae* antigen diminishes with the duration of the disease (Houba, 1975). Possible alternative mechanisms are outlined above. We do not know which apply in this disease. Similar chronic renal disease may also be associated with *P. falciparum* infec-

tion, particularly when it is resistant to chloroquine, thus providing a continual source of antigens for production of nephrotogenic IC (Boonpucknavig and Sitprija, 1979). This may explain the high prevalence of the nephrotic syndrome in adults in malaria areas, in whom QMN is uncommon, and in tropical areas where there is no *P. malariae*.

5.1.1b. Schistosomal Immune Complex Nephropathy

Schistosomal antigens, particularly *Schistosoma mansoni* and *S. japonicum*, probably cause IC-induced nephritis in man and other animals (Andrade *et al.*, 1971; Brito *et al.*, 1971; Von Lichtenberg *et al.*, 1972; Cavallo *et al.*, 1974; Natali and Cioli, 1976). A highly significant association of nephritis with *S. hematobium* infection has been reported (Ezzat *et al.*, 1974); others have detected circulating IC in some cases but without glomerulonephritis (Smith *et al.*, 1977; Sadigursky *et al.*, 1976), so the possibility of multifactorial etiology must be considered here.

There are more than 60 antigens in adult worms cercariae and schistosomula (Waksman and Cook, 1975), and the nephritogenic antigens are not known. They are likely to be accessible antigens such as tegument or intestinal lining (Andrade and Rocha, 1979). A high-molecular-weight polysaccharide antigen of intestinal lining has been identified in *S. mansoni* (Nash *et al.*, 1974; Nash, 1974). It is detected in serum (Berggren and Weller, 1967) and urine (Gold *et al.*, 1969). Low-molecular-weight polysaccharides have also been detected in serum and urine of heavily *S. mansoni*-infected hamsters as well as in secretory products of the adult worm (Deedler *et al.*, 1976). Their role in the pathogenesis of glomerular lesions is not known.

In man, M antigen has been found in the urine of infected patients (Carlier *et al.*, 1975), and other schistosomal antigens have been found in the milk of infected mothers (Santoro *et al.*, 1977). The eggs are another source of antigen, but it is not clear whether they are important in glomerulopathy in man. Animal experiments suggest that egg-derived antigens are indeed important in the pathogenesis of renal lesions in *S. mansoni* and *S. japonicum* infections, since mice infected with single-sex parasites (no egg production) had a much lower incidence of GN than those infected with male and female worms (Natali and Cioli, 1976). Much of the antibodies formed to egg antigens (Colley, 1975). Circulating egg antigens and antibodies, probably in complexed forms, were found in infected baboons, as were IC in which other schistosomal antigens were present (Houba *et al.*, 1976a).

Complexed immunoglobulin and complement have been demonstrated in the glomeruli in nephropathies associated with *S. mansoni* and *S. japonicum* infections in man and other animals (Cavallo *et al.*, 1974; Tada *et al.*, 1975; Andrade and Susin, 1974; Natali and Cioli, 1976; Van Marck *et al.*, 1977;

Falcão and Gould, 1975, Honshino-Shimizu *et al.*, 1976; World Health Organization, 1977). ImmunoglobulinG, IgM, IgE, occasionally IgA, and C3 were seen on immunofluorescence as granular deposits along the glomerular capillary basement membrane and mesangial areas. Electron-dense deposits were also seen in the subendothelial side of GBM and within the mesangium (Brito *et al.*, 1969). Antigens have not been demonstrated in the deposits in human cases, although they have in *S. mansoni*-infected mice (Natali and Cioli, 1976).

As further evidence of an immune-mediated pathogenesis in human schistosomal glomerulopathy, circulating IC and C3d fragments have been detected in sera of these patients (Lambert *et al.*, 1978; Verroust *et al.*, 1979), but *Schistosoma*-infected patients have varying complement depression and anti-complementary activity depending on the circulating antigen and antibody in the absence of nephritis (Madwar *et al.*, 1978). Histologically, the majority of human cases have diffuse proliferative glomerulonephritis, but mesangial cell proliferation may be the sole finding in early stages (Andrade and Rocha, 1979). Lobulation is marked in advanced disease. Other histopathological abnormalities seen include focal glomerulosclerosis, proliferative glomerulonephritis, and end-stage glomerular sclerosis (Andrade and Rocha, 1979). Classically, the disease occurs in young adults with hepatosplenomegaly whose first renal symptom is the nephrotic syndrome.

Nephropathy is limited to the hepatosplenic form of schistosomiasis. This may be because circulating IC and the polysaccharide antigens, which are normally cleared by the liver macrophages, bypass this organ through the collateral circulation and are deposited in the kidney (Van Marck *et al.*, 1977; Andrade and Rocha, 1979).

5.1.1c. Filarial Immune Complex Nephropathy

The role of IC of filarial origin in causing nephropathy has been recognized only recently.

i. Loa-loa. Glomerulonephritis has been described in patients with *Loa-loa* infection (Zuidema, 1971). The glomeruli showed thickening of the capillary loops but without any specific features. One patient with membranous nephritis associated with loiasis was reported (Bariéty *et al.*, 1967) in whom electron-dense deposits were noted in the epithelial surface, and another had loiasis and nephrotic syndrome (Pillay *et al.*, 1973). The evidence for IC-mediated renal damage of filarial origin was circumstantial in these cases. A Cameroonian adult male with heavy proteinuria, hypertension, and heavy *Loa-loa* infection had membranous glomerulonephritis (MGN), sclerosis of some glomeruli, and electron-dense deposits in the epithelial surface (Figure 2a) (Morley *et al.*, 1980). Immunofluorescence revealed deposits of IgG and C3 with a distribution typical of MGN. Using antisera to *Brugia pahangi*, which has common antigens with



Figure 2. a: Electron micrograph ($\times 10,000$) showing extensive dense deposits on epithelial surface of basement membrane with formation of basement membrane spikes between deposits. b: Immunoperoxidase staining of filarial antigen on basement membrane of typical glomerulus. (Photomicrographs reproduced by courtesy of Dr. A. R. Morley, Freeman Hospital, Newcastle-on-Tyne.)

Loa-loa, filarial antigens were detected in the complexes by immunoperoxidase staining (Figure 2b). These findings are in keeping with an IC pathogenesis in nephropathy associated with loiasis.

ii. *Onchocerca volvulus*. The antigens of this filarial worm are also complex and multiple. They include somatic and excretory/secretory substances (ESS) of the various larval stages and total more than 100 in the case of somatic products, and at least 10 in ESS (J. L. Ngu, unpublished data; Ngu *et al.*, 1980). Infected patients have complement-fixing IgM and IgG antibodies to worm antigens, high levels of immunoconglutinin (Ngu and Blackett, 1976), and circulating IC (World Health Organization, 1977; Lambert *et al.*, 1978), ranging in size from 1.5 to 4×10^6 daltons (Paganelli *et al.*, 1980). There is tentative evidence for two syndromes associated with IC nephropathy in this infection.

Acute Transient Nephropathy. Following treatment with diethylcarbamazine (DEC), large quantities of antigens are suddenly released into the blood, presumably to form complexes. Some of the patients have proteinuria,

often heavy, which disappears when treatment is stopped (Greene *et al.*, 1980; Ngu *et al.*, 1980). The histopathological and immunologic findings in the kidney are not known.

Chronic Persistent Nephropathy. In hyperendemic areas, some chronically infected patients develop proteinuria, sometimes with the nephrotic syndrome. In limited immunofluorescence studies, coarse granular deposits of immunoglobulin, mainly IgM but also IgA in some, and C3 have been seen along GBM (Figure 3). Using fluoresceinated antisera to somatic extracts of immature microfilariae of *O. volvulus* we identified antigen at sites of IC deposits in the glomeruli. These findings suggest deposition of nephritogenic complexes in the kidney. However, intact microfilariae are sometimes found in glomeruli (Figure 4) and in the urine following DEC treatment (Buck *et al.*, 1971), at which time proteinuria may occur. The glomerular worms could result in an Arthuslike deposition of immunoglobulin. In some cases, the nephropathy is chronic. Since the adult worms are not killed by DEC (the drug most commonly in use), an explanation of this could be continued antigen release.

5.1.1d. Other Parasites

Other parasitic infections may well cause nephropathy. Trypanosomiasis does so in other animals. Immune complex deposits have been demonstrated by

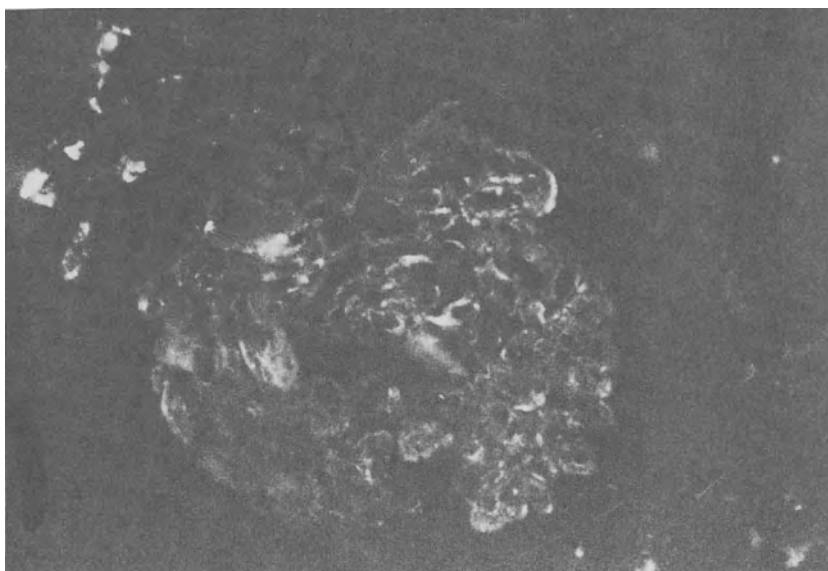


Figure 3. Photomicrograph of immunoglobulin deposition in glomeruli in *Loa-loa* nephropathy.

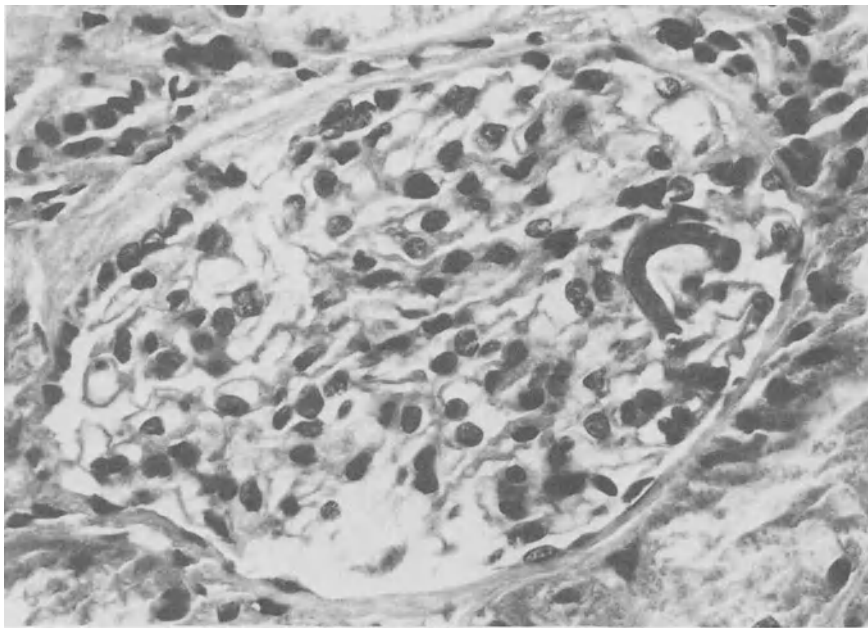


Figure 4. Microfilaria in the glomerulus of a patient with *O. volvulus* nephropathy (hematoxylin and eosin, $\times 530$). (Courtesy of Dr. Francois Chatelanat, Department of Pathology, Geneva.)

immunofluorescence and electron microscopy in kidneys of monkeys infected with *Trypanosoma rhodesiense* (Nagle *et al.*, 1974) with evidence of activation of complement by the alternative pathway. They also occur in mice infected with *T. brucei* (Lambert and Houba, 1974).

Toxoplasma gondii infections in man, particularly congenital cases, may also be associated with nephropathies (Wickbom and Winbert, 1972; Shahin *et al.*, 1974; Ginsburg *et al.*, 1974). Granular deposits of IgM and *T. gondii*-derived antigens in the glomerular capillary basement membrane and electron-dense deposits in the subendothelial region have been demonstrated in some of these cases.

5.1.2. Bacterial

The association of glomerulonephritis with certain bacterial infections well recognized in developed countries but now occurring less frequently because of improved hygiene persists in tropical countries. Early workers in the tropics failed to consider these possibilities and ascribed nephropathies they encountered exclusively to parasitic infections. This, indeed, was responsible for much of the

controversy that existed in the early reports on renal histological findings attributed to malarial nephropathy.

More precise diagnosis from detailed clinical evaluation led to the identification of the characteristic histology of, for instance, QMN (Hendrickse *et al.*, 1972). It would appear that the clinical manifestation of bacteria-associated nephropathy in the tropics is not the same as that in temperate regions, but we do not know the mechanisms of this difference.

5.1.2a. *Nephritogenic Streptococci*

Most acute glomerulonephritis is associated with streptococcal infection, particularly Group A, type 12, and some other types. Apart from deaths in the acute phase, the vast majority recover, but a few go on to chronic nephritis.

In temperate urban communities, the infection is usually respiratory, but the commonest site of nephritogenic streptococcal infection in the tropics is the skin, as was shown in outbreaks in Trinidad (Poon-King *et al.*, 1973). It has been reported in Nigeria as secondary to pyoderma following widespread scabies in school children sleeping close to each other during the wet season (Whittle *et al.*, 1973). The serum antibodies, which differ according to the site of infection, include antistreptolysin O, anti-DNase B, anti-NADase, antihyaluronidase, and anti-M protein (Wannamaker, 1970; Dillon, 1970). There is some evidence that complement may be activated through the classical or alternative pathway. Complexes of Ig and C are demonstrable as irregular granular deposits along GBM (Seegal *et al.*, 1965; McCluskey *et al.*, 1966; Fish *et al.*, 1970), and these disappear with resolution of the disease. However, streptococcal antigens are rarely detected in them. Electron-dense subepithelial deposits occur along GBM (Seegal *et al.*, 1965; Osawa *et al.*, 1966; Feldman *et al.*, 1966; Fish *et al.*, 1970; Yoshizawa *et al.*, 1973); these are complexes of Ig and antigen (Andres *et al.*, 1966). The histological picture is one of diffuse proliferation in the glomerulus in acute cases, with epithelial crescent formation which may progress to hyalinization in advanced cases.

The mechanisms involved in induction of kidney injury, although extensively studied, are still a subject of controversy. In those commonly cited, nephritogenic immune complexes are formed. This process persists after the overt infection, because constituents of the bacteria are retained in histocytes for months, providing antigen (Rickles *et al.*, 1969).

Streptococci, particularly Group A, share antigenic determinants with renal tissue (Treser *et al.*, 1970; Ahmed *et al.*, 1975; Blue and Lange, 1975a,b; Wheeler *et al.*, 1975); perhaps glomerular damage results from cross-reacting antibodies.

Streptococcal M protein binds directly to GBM where it complexes with fibrinogen and activates complement (Kantor, 1965 a, b).

Products of the bacteria may be directly toxic.

Serum protein constituents altered by the infection may cause the renal damage. Thus, rabbit serum incubated with streptococci forms a cryoglobulin which produces glomerular lesions with deposits of Ig and C3 but devoid of streptococcal products (McIntosh *et al.*, 1972).

It is also possible that cellular hypersensitivity may contribute to chronic damage in glomerulonephritis (Zabriskie, 1971; Macanovic *et al.*, 1972).

Although streptococci are the commonest cause of acute nephritis, other bacterial sources of nephritogenic IC have been reported in temperate regions and may equally apply to the tropics. These include *Streptococcus viridans* (Morel-Maroger *et al.*, 1972) and *Staphylococcus albus* (Tu *et al.*, 1969) in bacterial endocarditis, *Treponema pallidum* (Falls *et al.*, 1965; Braunstein *et al.*, 1970; Hellier *et al.*, 1971; Hill *et al.*, 1972; Kaplan *et al.*, 1972), and *Salmonella typhosa* (Sitprija *et al.*, 1974).

5.1.2b. Leprosy (*Mycobacterium leprei*)

Leprosy, a common infection in many tropical countries, is associated with IC glomerulonephritis in some cases, but the incidence is unknown. Granular deposits of Ig and C are found in the glomeruli (Shwe, 1971; Bullock *et al.*, 1974; Date and Johnny, 1975; Iveson *et al.*, 1975). In a recent study of 20 renal biopsies from patients with borderline lepromatous or lepromatous leprosy, nine had mesangial proliferative GN, one rapidly progressive GN, one amyloidosis, one chronic pyelonephritis, and eight normal renal histology (Cöloglu, 1979). All of the patients with mesangial proliferative glomerulonephritis had granular deposits containing IgG, IgM, and C3, and two had IgA. Electron-dense deposits were found in the mesangium and occasionally in the basement membrane.

5.1.3. Viral

Chronic viral infections may cause chronic IC glomerulonephritis in animals (Oldstone, 1975), e.g., chronic lymphocytic choriomeningitis (LCM) virus infection (Oldstone and Dixon, 1969, Oldstone *et al.*, 1973). Immunoglobulin G antibody, virus antigen, and C are deposited in the glomerular capillaries and basement membrane. Examples in other species include Aleutian mink disease, murine leukemia virus, lactic dehydrogenase virus, and equine infectious anemia virus (Leader, 1971; Wilson and Dixon, 1976). A common denominator in all of these infections is persistent release of viral antigens which form IC. The antigen may be the entire virion or portions of it or neoantigens induced by the viral invasion.

There is evidence of human analogues of this type of IC nephropathy. Glomerular lesions in man have been reported in association with the following: Epstein-Barr virus in Burkitt's lymphoma (Oldstone *et al.*, 1974) and infectious mononucleosis (Wilson and Dixon, 1976), measles virus in subacute sclerosing panencephalitis, mumps, varicella, dengue fever (Wilson and Dixon, 1976), and hepatitis B virus (Schulman and Barker, 1969; Combes *et al.*, 1971; Kohler *et al.*, 1974; Brzoski *et al.*, 1974; Ozawa *et al.*, 1976; Slusarczyk *et al.*, 1980). Hepatitis B virus (HBV)-associated IC nephropathy deserves special mention, as this infection presents a grave public health problem in many tropical countries where the HBV antigenemia exceeds 10% among blood donors (Lancet, 1980). In Cameroon we have noted a prevalence of 9% in random blood samples taken from subjects in and around Yaoundé. Hepatitis B surface antigen (HBsAg) and core antigen (HBcAg) are the major antigenic components of this virus (Almeida and Waterson, 1969; Almeida *et al.*, 1971; Brzosko *et al.*, 1973; Hoofnagle *et al.*, 1973). In the acute phase, extrahepatic symptoms resembling acute serum sickness (e.g., urticaria and joint pain) may be seen in about a fifth of cases before overt hepatitis develops. Transient glomerulonephritis, cutaneous vasculitis, and alteration of serum C components (including low C4 levels which indicate classical pathway activation) occur (Kohler, 1973). Granular deposits of immunoglobulin and C and electron-dense deposits occur along GBM (Combes *et al.*, 1971; Randall *et al.*, 1971; Eknoyam *et al.*, 1972; Brzoski *et al.*, 1974; Slusarczyk *et al.*, 1980). Recently, Slusarczyk and collaborators (1980) reported that of 98 Polish children with the nephrotic syndrome, 24 had evidence of HBV infection and IC deposits of HBV antigen, immunoglobulin, and C in renal biopsies. Twenty-one had diffuse membranous glomerulonephritis (MGN). Hepatitis B virus core antigen was the sole antigen in a third of cases. Both core and surface antigens were found in another third, and HBV surface antigen alone in the remaining third of cases.

5.1.4. Noninfective Agents

A growing number of agents derived from noninfective sources are associated with IC renal damage. These may be drugs such as procainamide, hydralazine, trimethadone, penicillamine, sulfa compounds, gold and mercurial compounds, and probably other chemical agents. They cause GN with IC deposits demonstrable by immunofluorescence and/or electron microscopy. The mechanisms may be antibody to either the drug alone or haptogenic conjugates of the drug or its metabolites. Release of endogenous antigens through the toxic effect of the drug or chemical may also occur. For instance, captopril is associated with MGN, IC deposits in the kidney, and the production of antibodies against nuclear proteins or double-stranded DNA (Prins *et al.*, 1979; Hoorntje *et al.*, 1980). The

acute interstitial nephritis associated with diflunisal has C3, IgM, and IgG deposited along the tubular basement membranes (Chan *et al.*, 1980).

The nephrotic syndrome in some Kenyan women was traced to the use of a mercurial skin-lightening cream (Barr *et al.*, 1972). Hydrocarbon derivatives have also been incriminated (Zimmerman *et al.*, 1975; Von Scheele *et al.*, 1976; Cagnoli *et al.*, 1980).

5.2. Endogenous Antigens

Endogenous antigens induce the formation of nephritogenic IC in man and other animals. Systemic lupus erythematosus (SLE) is the best-studied example; exacerbations caused by sunlight are particularly severe in whites in tropical climates.

5.2.1. Nuclear Antigens

Systemic lupus erythematosus results from a complex interaction of genetic and environmental factors; there are dysfunctions of both the cellular and humoral immune systems (Koffler, 1980). Evidence for genetic predisposition comes from the higher concordance in identical twins than in fraternal twins, the increased prevalence in first-degree relatives of SLE patients than in the general population, and the association with certain histocompatibility types. It is also associated with various complement defects (Lachmann, 1975) and with heterozygosity for chronic granulomatous disease (Thompson and Soothill, 1970). Environmental factors include drugs such as procainamide and hydralazine. Other environmental agents that affect it are ultraviolet irradiation and RNA viruses of the C type (Koffler, 1980). Deficient suppressor T-cell function in patients and their relations (Miller and Schwartz, 1979) or enhanced helper T-cell proliferation causing unbridled B-lymphocyte activity may contribute to the disease.

Sera from SLE patients have antibodies to native and single-stranded DNA, nucleoproteins, and other nuclear constituents. Cytotoxic and other anticellular antibodies may also be produced. Activation of both classical and alternate pathway components occurs in these cases, and circulating IC are readily detectable.

Immune complex deposits in the kidney consist of IgG, IgA, IgM, and both early- and late-acting C components, properdin and C3 (Wilson and Dixon, 1976). They are found first in the mesangial cells and later in the GBM as diffuse fine or coarse granules. The antigens in these complexes have been identified as both native and single-stranded DNA (Koffler *et al.*, 1967, 1974; Andres *et al.*, 1970).

5.2.2. Renal Tubular Antigens

Antigens in the brush border of the proximal convoluted tubule may cause nephritis. This nephritis occurs in sickle cell anemia and so is of particular tropical importance (Wilson and Dixon, 1976).

5.2.3. Tumor Antigens

Certain tumors of man and experimental animals are associated with nephritis caused by nephritogenic IC-containing tumor antigens (Wilson and Dixon, 1976).

6. TREATMENT

6.1. Management

Such transient IC nephropathy as poststreptococcal acute glomerulonephritis and that which occurs in *P. falciparum* infections or following treatment of *O. volvulus* should always be distinguished from the chronic persistent nephropathy, and treatment should be antiparasitic and supportive. Since such etiological factors are so common in the tropics, the coexistence of more than one in the same patient should always be considered. In chronic nephritis, treatment of the causative agent, although effective in nephritis caused by *Treponema pallidum*, is rarely effective in the others. It should be given, however.

6.2. Immunosuppressive and Antiinflammatory Treatment

Apart from the use of steroids in steroid-responsive nephrotic syndrome, antiinflammatory and immunosuppressive drugs have been disappointing in glomerulonephritis. Steroid treatment may be disastrous, especially in children in tropical countries, if used arbitrarily, as there is risk of hypertension, encephalopathy, severe infections, and sudden death in patients with QMN (Hendrickse and Gilles, 1963; Hendrickse, 1966; Adeniyi *et al.*, 1976). The minority of patients with nephrotic syndrome likely to benefit can be identified by minimal changes on renal biopsy or a highly selective proteinuria of differential protein clearances (Soothill and Hendrickse, 1967; Adeniyi *et al.*, 1976). Remission in some children with mild (grade 1) QMN was interesting. Remission sometimes followed combined steroid and cyclophosphamide in some adult Nigerians with proliferative GN, but it is not clear whether this is causative (Ngu and Monekosso, 1968; Ngu, 1969; Ngu and Blackett, 1970). This was seen in a few

patients with QMN (Adeniyi *et al.*, 1979), but in a controlled trial of cyclophosphamide and azathioprine in QMN, these authors showed that, whereas the former had no systematic effect, the latter significantly accelerated the onset of chronic renal failure. Cytotoxic drugs are acutely toxic, although there were no deaths from this in this study, and azathioprine can make the nephritis worse, so they should not be used lightly.

6.3. Possible Future Lines of Treatment

From what has been said, there are already several other possible sites of intervention and prevention of continual renal damage. Despite the failure to induce remission in QMN by eliminating the parasite with drugs (Hendrickse and Adeniyi, 1979), avoidance of the causative agent is important. Besides the therapeutic effect in some infections such as syphilis, it is important prophylactically in view of the dramatic reduction in the incidence of nephropathy in Guyana when malaria was eradicated (Giglioli, 1962). Such effects may also be possible in viral nephritis. In addition to hygiene to control hepatitis virus, treatment may be possible. We gave a nephrotic patient who was unresponsive to steroid therapy and who had MGN and persistent HBsAg in her blood two doses of antihepatitis B immunoglobulin, steroids, indomethacin, and heparin; for 7 months since, she has been free of edema, proteinuria, and HBsAg. Recently, Tabor *et al.* (1980) reported that HBV infectivity can be removed from factor-IX complex using B-immune globulin. More studies are urgently needed to evaluate this therapeutic approach to HBV nephropathy, as the use of long-term prednisone and/or azathioprine advocated by some may enhance the replication of the virus (Sagnelli *et al.*, 1980). Alternatively, (+)-cyanidanol-3, which demonstrably accelerates the disappearance of HBsAg from the blood in acute viral hepatitis (Blum *et al.*, 1977, DiNola, 1980), may be tried in nephropathy associated with this infection.

Manipulation of the antibody response by altering the type, quality or quantity of antibody is another possible method of intervention (World Health Organization, 1977), as is removal of IC by plasmapheresis, as has been attempted in SLE, or other measures (World Health Organization, 1977). Attempts to block the phlogistic effects of the activated complement fragments, mediators, and damage caused by the release of proteolytic enzymes from effector cells are still experimental (World Health Organization, 1977).

7. CONCLUSIONS

There are still large gaps in our knowledge of IC nephropathy which is common and important in many tropical countries. Identification of the many

etiologic agents may lead to economically feasible prevention. The role of viruses, hepatitis B in particular, needs particular study. With greater understanding of the interaction of such environmental factors as malnutrition, some prevention may be possible before radical prevention by provoking infections becomes feasible.

The therapeutic effectiveness of antigen elimination, blocking mediator mechanisms of IC-induced damage or effector cells, has been disappointing so far but needs more study in the different etiologic forms of nephropathies. Such community preventive measures as immunization or hygiene for malaria and hepatitis B virus infection offer the best hope for the future.

REFERENCES

- Adeniyi, A., Hendrickse, R. G., and Soothill, J. F., 1976, Differential protein clearances and response to treatment in Nigerian nephrotic children, *Arch. Dis. Child.* 51:691.
- Adeniyi, A., Hendrickse, R. G., and Soothill, J. F., 1979, A controlled trial of cyclophosphamide and azathioprine in Nigerian children with the nephrotic syndrome and poorly selective proteinuria, *Arch. Dis. Child.* 54:204.
- Ahmed, U., Lange, K., and Treser, G., 1975, Relationship between naturally and experimentally produced antibodies to streptococcal nephritogen, *Fed. Proc.* 34:3726.
- Allison, A. C., Houba, V., Hendrickse, R. G., DePetris, S., Edington, G. M., and Adeniyi, A., 1969, Immune complexes in the nephrotic syndrome of African children, *Lancet* 1:1232.
- Almeida, J. D., Rubenstein, D., and Stott, E. J., 1971, New antigen-antibody system in Australia-antigen positive hepatitis, *Lancet* 2:1225.
- Almeida, T. D., Waterson, A. P., 1969, Immune-complexes in hepatitis, *Lancet* 2:983-986.
- Alpers, J. H., Steward, M. W., and Soothill, J. F., 1972, Differences in immune elimination in inbred mice. The role of low affinity antibody, *Clin. Exp. Immunol.* 12:121.
- Andradé, Z. A., and Rocha, H., 1979, Schistosomal glomerulopathy, *Kidney Int.* 16:23.
- Andradé, Z. A., and Susin, M., 1974, Renal changes in mice infected with *Schistosoma mansoni*, *Am. J. Trop. Med. Hyg.* 23:400.
- Andradé, Z. A., Andradé, S. G., and Sadigursky, M., 1971, Renal changes in patients with hepatosplenic schistosomiasis, *Am. J. Trop. Med. Hyg.* 20:77.
- Andres, G. A., Accini, L., Hsu, K. C., Zabriskie, J. B., and Seegal, B. C., 1966, Electron microscopic studies of human glomerulonephritis with ferritin conjugated antibody. Localization of antigen-antibody complexes in glomerular structures of patients with acute glomerulonephritis, *J. Exp. Med.* 123:399.
- Andres, G. A., Accini, L., Beiser, S. M., Christian, C. L., Cinotti, G. A., Erlanger, B. F., Hsu, K. C., and Seegal, B. C., 1970, Localization of fluorescein-labeled antinucleoside antibodies in glomeruli of patients with active systemic lupus erythematosus nephritis, *J. Clin. Invest.* 49:2106.
- Austen, K. F., 1974, Hageman factor-dependent coagulation, fibrinolysis and kinin generation, *Transplant. Proc.* 6:39.
- Bariéty, J., Barbier, M., Laigre, M. C., Tcherman, G., Samarcq, P., and Milliez, P., 1967, Protéinurie et loase: Etude histologique optique et electronique d'un cas, *Bull. Soc. Med. (Paris)* 118:1015.
- Barr, R. D., Rees, P. H., Cordy, P. E., Kung'u, A., Woodger, B. A., and Cameron, H. M., 1972, Nephrotic syndrome in adult Africans in Nairobi, *Br. Med. J.* 2:131.

- Berger, M., Birch, L. M., and Conte, N. F., 1967, The nephrotic syndrome secondary to acute glomerulonephritis during *falciparum* malaria. *Ann. Intern. Med.* 67:1163.
- Berger, J., Noel, L. H., and Yanerva, T., 1974, Complement deposition in the kidney, *Adv. Nephrol.* 4:37.
- Berggren, W. L., and Weller, T. H., 1967, Immuno-electrophoretic demonstration of specific circulating antigen in animals infected with *Schistosoma mansoni*, *Am. J. Trop. Med. Hyg.* 16:606.
- Bhamarapravati, N., Boonpucknavig, S., Boonpucknavig, V., and Yaeboonruang, C., 1973, Glomerular changes in acute *Plasmodium falciparum* infection: An immunologic study, *Arch. Pathol.* 96:289.
- Blue, W. T., and Lange, C. F., 1975a, Increased immunologic reactivity between human glomerular basement membrane and Group A streptococcal cell membrane after carbohydrase treatment, *J. Immunol.* 114:306.
- Blue, W. T., and Lange, C. F., 1975b, Survey of antistreptococcal cell membrane (SCM) sera for antiglomerular basement membrane (GBM) activity, *Fed. Proc.* 34:4531.
- Blum, A. L., Berthet, P., Doelle, W., Goebell, H., Kortüm, K., Pelloni, S., Peter, P., Poulsen, H., Strohmeyer, G., and Tygstrup, N., 1977, Treatment of acute viral hepatitis with (+)-cyanidanol-3, *Lancet* 2:1153.
- Bokisch, V. A., Müller-Eberhard, H. J., and Cochrane, C. G., 1969, Isolation of a fragment (C3a) of the third component of human complement containing anaphylatoxin and chemotactic activity and description of an anaphylatoxin inactivator of human serum, *J. Exp. Med.* 129:1109.
- Boonpucknavig, V., and Sitprija, V., 1979, Renal disease in acute *Plasmodium falciparum* infection in man, *Kidney Int.* 16:44.
- Bout, D., Santoro, F., Carlier, Y., Bina, J. C., and Capron, A., 1977, Circulating immune complexes in schistosomiasis, *Immunology* 32:17.
- Braunstein, G. D., Lewis, E. J., Galvanek, E. G., Hamilton, A., and Bell, W. R., 1970, The nephrotic syndrome associated with secondary syphilis. An immune deposit disease, *Am. J. Med.* 48:643.
- Brito, T., Boni, D., Lopes, J. D., and Silva L. C., 1969, Kidney biopsy in human schistosomiasis: An ultrastructural study; (preliminary report), *Rev. Inst. Med. Trop. Sao Paulo* 11:62.
- Brito, T., Gunji, J., Camargo, M. E., Caravolo, A., and Silva, L. C., 1971, Glomerular lesions in experimental infections of *Schistosoma mansoni* in *Cebus apella* monkeys, *Bull. WHO* 45:419.
- Brzosko, W. J., Krawczynski, K., Nazarewicz, T., Morzycka, M., and Nowoslawski, A., 1974, Glomerulonephritis associated with hepatitis-B surface antigen immune complexes in children, *Lancet* 2:478.
- Brzosko, W. T., Madalinski, K., Krawczynski, K., and Nowoslawski, A., 1973, Duality of hepatitis-B antigen and its antibody: I. Immunofluorescence studies, *J. Infect. Dis.* 127:424.
- Buck, A. A., Anderson, R. I., Conston, J. A. C., Jr., Wallace, C. K., Connor, D. R., Harman, L. E., Jr., Donner, M. V., and Ganley, J. P., 1971, Microfilaruria in onchocerciasis. A clinical and epidemiological follow-up study in the Republic of Chad, *Bull. WHO* 45:353.
- Bullock, W. E., Callera, M. L., and Panner, B. J., 1974, Immunohistologic alteration of skin and ultrastructural changes of glomerular basement membrane in leprosy, *Am. J. Trop. Med. Hyg.* 23:81.
- Cagnoli, L., Casanova, S., Pasquali, S., Bonini, U., and Zucchelli, P., 1980, Relation between hydrocarbon exposure and the nephrotic syndrome, *Br. Med. J.* 280:1068.
- Cameron, J., and Kibukamusoke, J. W., 1971, Plasma C3 concentrations in malarial nephropathy, *East Afr. Med. J.* 48:466.
- Carlier, Y., Bout, D., Bina, J. C., Camus, D., Figueredo, J. F. M., and Capron, A., 1975, Immunological studies in human schistosomiasis: I. Parasitic antigen in urine, *Am. J. Trop. Med. Hyg.* 24:949.

- Cavallo, T., Galvanek, E. G., Ward, P. A., and von Lichtenberg, F., 1974, The nephropathy of experimental hepatosplenic schistosomiasis, *Am. J. Pathol.* 76:433.
- Chan, L. K., Winearls, C. G., Oliver, D. O., and Dunnill, M. S., 1980, Acute interstitial nephritis and erythroderma associated with diflunisal, *Br. Med. J.* 280:84.
- Cochrane, C. G., Revak, S. D., Aiken, B. S., and Wuepper, K. D., 1972, The structural characteristics and activation of Hageman Factor, in: *Inflammation Mediation and Control*, I. H. Lepow and P. A. Ward eds., Academic Press, New York, p. 19.
- Colley, D. G., 1975, Immune responses to a soluble schistosomal egg antigen preparation during chronic primary infection with *S. mansoni*, *J. Immunol.* 115:150.
- Cöglöglu, A. S., 1979, Immune complex glomerulonephritis in leprosy, *Lepr. Rev.* 50:213.
- Combes, B., Stastny, P., Shorey, J., Eigenbrodt, E. H., Barrera, A., Hull, A. R., and Carter, N. W., 1971, Glomerulonephritis with deposition of Australia antigen-antibody complexes in glomerular basement membrane, *Lancet* 2:234.
- Coovadia, H. M., and Soothill, J. F., 1976, The effect of amino-acid restricted diets on the clearance of ¹²⁵I-labelled polyvinyl pyrrolidone in mice, *Clin. Exp. Immunol.* 23:562.
- Date, A., and Johnny, K. V., 1975, Glomerular subepithelial deposits in lepromatous leprosy, *Am. J. Trop. Med. Hyg.* 24:853.
- Deedler, A. M., Klappe, H. T. M., Aardweg, G. J. M. J., and Meerbeke, E. H. E. M., 1976, *Schistosoma mansoni*: Demonstration of two circulating antigens in infected hamsters, *Exp. Parasitol* 40:189.
- Devey, M. E., and Steward, M. W., 1980, The induction of chronic antigen-antibody complex disease in selectively bred mice producing either high or low affinity antibody to protein antigens, *Immunology* 41:303.
- DiNola, F., 1980, (+)-Cyanidanol-3 in acute viral hepatitis, *Lancet* 2:1379.
- Dillon, H. C., Jr., 1970, Streptococcal skin infection and acute glomerulonephritis, *Postgrad. Med. J.* 68:641.
- Dixon, F. J., Feldman, J. P., and Vazquez, J. J., 1961, Experimental glomerulonephritis, *J. Exp. Med.* 113:899.
- Eknoyan, G., Györkey, F., Dichoso, C., Martinez-Maldonado, M., Suki, W. N., and Györkey, P., 1972, Renal morphological and immunological changes associated with acute viral hepatitis, *Kidney Int.* 1:413.
- Ezzat, E., Osman, R. A., Ahmet, K. Y., and Soothill, J. F., 1974, The association between *schistosoma haematobium* infection and heavy proteinuria, *Trans. R. Soc. Trop. Med. Hyg.* 68:315.
- Falcoa, H. A., and Gould, D. P., 1975, Immune complex nephropathy in schistosomiasis, *Ann. Intern. Med.* 83:148.
- Falls, W. F., Jr., Ford, K. L., Ashworth, C. T., and Carter, N. W., 1965, The nephrotic syndrome in secondary syphilis: Report of a case with renal biopsy findings, *Ann. Intern. Med.* 63:1047.
- Fearon, D. T., Ruddy, S., Knotsman, J. D., Carpenter, C. B., and Austen, K. F., 1974, The functional significance of complement, *Adv. Nephrol* 4:15.
- Feldman, J. D., Mardiney, M. R., and Shuler, S. E., 1966, Immunology and morphology of acute post-streptococcal glomerulonephritis, *Lab. Invest.* 15:283.
- Fish, A. J., Herdman, R. C., Michael, A. F., Pickering, R. J., and Good, R. A., 1970, Epidemic acute glomerulonephritis associated with type 49 streptococcal pyoderma. II. Correlative study of light, immunofluorescent and electron microscopic findings, *Am. J. Med.* 48:28.
- Fisher, E. R., and Bark, J., 1961, Effect of hypertension on vascular and other lesions of serum sickness, *Am. J. Pathol.* 39:665.
- Futrakul, P., Boonpucknavig, B., Boonpucknavig, S., Mittrakul, C., and Bhamarapravati, N., 1974, Acute glomerulonephritis complicating *Plasmodium falciparum* infection, *Clin. Pediatr.* 13:281.

- Gelfand, M. C., Frank, M. M., and Green I., 1975, A receptor for the third component of complement in the human renal glomerulus, *J. Exp. Med.* 142:1029.
- Germuth, F. G., Jr., and Rodriguez, E., 1973, *Immunopathology of the Renal Glomerulus. Immune-Complex Deposit and Antibasement Membrane Disease*, Little, Brown, Boston, 1973.
- Germuth, F. G., Jr., Kelemen, W. A., and Pollack, A. D., 1967, Immune complex disease, II. The role of circulatory dynamics and glomerular filtration in the development of experimental glomerulonephritis, *Johns Hopkins Med. J.* 120:252.
- Giglioli, G., 1962, Malaria and renal disease with special reference to British Guyana II: The effect of malarial eradication on the incidence of renal disease in British Guyana, *Ann. Trop. Med. Parasitol.* 56:225.
- Gilles, H. M., and Hendrickse, R. G., 1960, Possible aetiological role of *Plasmodium malariae* in 'nephrotic syndrome' in Nigerian children, *Lancet* 1:806.
- Ginsburg, B. E., Wasserman, J., Hultdt, G., and Bergstrand, A., 1974, A case of glomerulonephritis associated with acute toxoplasmosis, *Br. Med. J.* 3:664.
- Gold, R., Rosen, R. S., and Weller, T. H., 1969, Specific circulating antigen in hamsters infected with *Schistosoma mansoni*: Detection of antigen in serum and urine, and correlation between antigenic concentration and worm burden, *Am. J. Trop. Med. Hyg.* 18:545.
- Greene, B. M., Taylor, H. R., and Humphrey, R. L., 1980, Proteinuria associated with diethylcarbamazine treatment of onchocerciasis, *Lancet* 1:254.
- Hellier, M. D., Webster, A. D. B., and Eisinger, A. J. M. F., 1971, Nephrotic syndrome: A complication of secondary syphilis, *Br. Med. J.* 4:404.
- Hendrickse, R. G., 1966, in: *Proceedings of the 3rd International Congress of Nephrology* (G. E. Schreiner ed.), Karger, Basel, p. 208.
- Hendrickse, R. G., and Adeniyi, A., 1979, Quartan malarial nephrotic syndrome in children, *Kidney Int.* 16:64.
- Hendrickse, R. G., and Gilles, M., 1963, The nephrotic syndrome and other renal diseases in children in Western Nigeria, *East Afr. Med. J.* 40:186.
- Hendrickse, R. G., Adeniyi, A., Edington, G. M., Glasgow, E. F., White, R. H. R., and Houba, V., 1972, Quartan malarial nephrotic syndrome: Collaborative clinico-pathological study in Nigerian Children, *Lancet* 1:1143.
- Henson, P. M., 1969, The adherence of leukocytes and platelets induced by fixed IgG antibody or complement, *Immunology* 16:107.
- Hill, L. L., Singer, D. B., Falleta, J., and Stasney, R., 1972, The nephrotic syndrome in congenital syphilis: An immunopathology, *Pediatrics* 49:260.
- Honshino-Shimizu, S., Brito, T., Kanamura, H. Y., Canto, A. L., Silva, A. O., Campos, A. R., Penna, D. O., and Silva, L. C., 1976, Human schistosomiasis: *Schistosoma mansoni* antigen detection in renal glomeruli, *Trans. R. Soc. Trop. Med. Hyg.* 70:492.
- Hoofnagle, T. H., Gerety, R. T., and Barker, L. F., 1973, Antibody to hepatitis-B-virus core antigen in man, *Lancet* 2:869.
- Hoorntje, S. J., Kallenberg, C. G. M., Weening, J. J., Donker, A. J. M., The, T. H., and Hoedemaeker, P. J., 1980, Immune complex glomerulopathy in patients treated with captopril, *Lancet* 1:1212.
- Houba, V., 1975, Immunopathology of nephropathies associated with malaria, *Bull. WHO* 52:199.
- Houba, V., 1979, Immunologic aspects of renal lesions associated with malaria, *Kidney Int.* 16:3.
- Houba, V., and Lambert, P. H., 1974, Immunological studies on tropical nephropathies, *Adv. Biosci.* 12:617.
- Houba, V., and Williams, A. I. O., 1972, Soluble serum antigens of *P. falciparum* in Nigerians. II. Immunochemical studies, *Afr. J. Med. Sci.* 3:309.
- Houba, V., Allison A. C., Hendrickse, R. G., DePetris, S., Edington, G. M., and Adeniyi, A.,

- 1970, Immune complex in the nephrotic syndrome of Nigerian children, in: *Proceedings International Symposium of Immune Complex Diseases*, L. Bonomo and J. Li Turk, eds. Carlo Erba Foundation, Milan, pp.23–35.
- Houba, V., Allison, A. C., Adeniyi, A., and Houba, J. E., 1971, Immunoglobulin classes and complement in biopsies of Nigerian children with the nephrotic syndrome, *Clin. Exp. Immunol.* 8:761.
- Houba, V., Koech, D. K., Sturrock, R. F., Butterworth, A. E., Kusel, J. R., and Mahmoud, A. A. F., 1976a, Soluble antigens and antibodies in sera from baboons infected with *S. mansoni*, *J. Immunol.* 117:705.
- Houba, V., Lambert, P. H., Voller, A., and Soyawo, M. A. O., 1976b, Clinical and experimental investigation of immune complexes in malaria, *Clin. Immunol. Immunopathol.* 6:1.
- Hunsicker L. G., 1974, Abnormalities of the complement system in acquired renal disease, *Transplant. Proc.* 6:77.
- Hyman, L. R., Jenis E. H., Hill, G. S., Zimmerman, S. W., and Burkholder, P. M., 1975, Alternate C3 pathway activation in pneumococcal glomerulonephritis, *Am. J. Med.* 58:810.
- Iveson, J. M. I., McDougal, A. C., Leatham, A. J., and Harris, H. J., 1975, Lepromatous leprosy presenting with polyarthritis, myositis and immune complex glomerulonephritis, *Br. Med. J.* 3:619.
- Izui, S., Lambert, P. H., and Miescher, P. A., 1976, *In vitro* demonstration of a particular affinity of glomerular basement membrane and collagen for DNA. A possible basis for a local formation of DNA–anti-DNA complexes in systemic lupus erythematosus, *J. Exp. Med.* 144(2):428.
- Jenis, E. H., Sandler, P., Hill, G. S., Knieser, M. R., Jenson, G. E., and Roskes, S. D., 1974, Glomerulonephritis with basement membrane dense deposits, *Arch. Pathol.* 97:84.
- Kantor, F. S., 1965a, Fibrinogen precipitation by streptococcal M Protein. I. Identity of the reactants, and stoichiometry of the reaction, *J. Exp. Med.* 121:849.
- Kantor, F. S., 1965b, Fibrinogen precipitation by streptococcal M protein. II. Renal lesions induced by intravenous injection of M protein into mice and rats, *J. Exp. Med.* 121:861.
- Kaplan, A. P., and Austen, K. F., 1970, A pre-albumin activator of prekallikrein, *J. Immunol.* 105:802.
- Kaplan, B. S., Wigglesworth, F. W., Marks, M. I., and Drummond, K. N., 1972, The glomerulopathy of congenital syphilis—an immune deposit disease, *J. Pediatr.* 81:1154.
- Kibukamusoke, J. W., 1966, *The Nephrotic Syndrome in Uganda*, M.D. Thesis, University of East Africa, Kampala.
- Kibukamusoke, J. W., Hutt, M. S. R., and Wilks, N. E., 1967, The nephrotic syndrome in Uganda and its association with quartan malaria, *Q. J. Med.* 36:393.
- Kincaid-Smith, P., 1972, Coagulation and renal disease, *Kidney Int.* 2:183.
- Koffler, D., 1980, Systemic lupus erythematosus: This serious rheumatic disease, found largely among women, is a disease of the immune system, *Sci. Am.* 243:40.
- Koffler, D., Schur, P. H., and Kunkel, H. G., 1967, Immunological studies concerning the nephritis of systemic lupus erythematosus, *J. Exp. Med.* 126:607.
- Koffler, D., Agnello, V., and Kunkel, H. G., 1974, Polynucleotide immune complexes in serum and glomeruli of patients with systemic lupus erythematosus, *Am. J. Pathol.* 74:109.
- Kohler, P. F., 1973, Clinical immune complex disease: Manifestations in systemic lupus erythematosus and hepatitis B virus infection, *Medicine (Baltimore)* 52:419.
- Kohler, P. F., Cronin, R. E., Hammond, W. S., Olin, D., and Carr, R. I., 1974, Chronic membranous glomerulonephritis caused by hepatitis B antigen–antibody immune complexes, *Ann. Intern. Med.* 81:448.
- Lachmann, P. J., 1975, Complement, in: *Clinical Aspects of Immunology*, (P. G. W. Gell, R. R. A. Coombs, and P. J. Lachmann, eds.), Blackwell, Oxford.
- Lambert, P. H., and Houba, V., 1974, Immune complexes in parasitic diseases, in: *Progress in*

- Immunology*: II. Volume 5. L. Brent and J. Holborow, eds., North Holland, Amsterdam, pp. 57–67.
- Lambert, P. H., Perrin, L. H., Mahieu, P., and Miescher, P. A., 1973, Immunopathology of the C3 activation systems, in: *Immunopathology*, Volume VII, *Advances in the Biosciences*, cavtat, p. 281.
- Lambert, P. H., Perrin, L. H., Mahieu, P., Nydegger, U. E., and Miescher, P. A., 1974, Activation of complement in human nephritis, *Adv. Nephrol.* 4:79.
- Lambert, P. H., Dixon, F. J., Zubler, R., Agnello, V., Cambiaso, C., Casali, P., Clarke, J., Cowdery, J. S., Frederic, M. D., McDuffie, C., Hay, F. C., MacLennan, I. C. M., Masson, P., Müller-Eberhard, H. J., Pentimen, K., Smith, M., Tappeiner, G., Theofilopoulos, A. N., Verroust, P., 1978, A WHO collaborative study for the evaluation of eighteen methods for detecting immune complexes in serum, *J. Clin. Lab. Immunol.* 1:1.
- Lancet*, 1980, Nephrotic syndrome in the Tropics, *Lancet*, 2:461.
- Leader, R. W., 1971, Viruses and autoimmune diseases in animals other than man, *Am. J. Clin. Pathol.* 56:315.
- Leber, P. D., and McCluskey, R. T., 1974, Complement and immunohistology of renal disease, *Transplant. Proc.* 6:67.
- Levinsky, R. J., and Barratt, T. M., 1979, IgA immune complexes in Henoch–Schönlein purpura, *Lancet* 2:1100.
- Levinsky, R. J., Cameron, J. S., and Soothill, J. F., 1977, Serum immune complexes and disease activity in lupus nephritis, *Lancet* 1:564.
- Linscott, W. D., and Nishioka, K., 1963, Components of guinea pig complement. II. Separation of serum fractions essential for immune hemolysis, *J. Exp. Med.* 118:795.
- Macanovic, M., Evans, D. J., and Peters, D. K., 1972, Allergic response to glomerular basement membrane in patients with glomerulonephritis, *Lancet* 2:207.
- Madwar, M. A., O'Shea, J. M., Skelton, J. A., and Soothill, J. F., 1978, Complement components and immunoglobulins in patients with schistosomiasis, *Clin. Exp. Immunol.* 34:354.
- Mannik, M., and Arend, W. P., 1971, Fate of preformed immune complexes in rabbits and rhesus monkeys, *J. Exp. Med.* 134:19s.
- Marder, V. J., 1971, Fibrinogen and fibrin degradation products. Physicochemical and physiologic considerations, *Thromb. Diath. Haemorr. [Suppl.]* 47:85.
- McCluskey, R. T., Vassalli, P., Gallo, G., and Baldwin, D. S., 1966, An immunofluorescent study of pathogenic mechanisms in glomerular disease, *N. Engl. J. Med.* 274:695.
- McGregor, I. A., Turner, M. W., Williams, K., and Hall, P., 1968, Soluble antigens in the blood of African patients with severe *Plasmodium falciparum* malaria, *Lancet* 1:881.
- McIntosh, R. M., Kaufman, D. B., Griswold, W., Smith, F. G., and Vernier, R. L., 1971, Glomerular localization of fibrinogen—clinicopathologic, prognostic and therapeutic considerations, *J. Chronic. Dis.* 24:787.
- McIntosh, R. M., Kaufman, D. B., McIntosh, J. R., and Griswold, W., 1972, Glomerular lesions produced by autologous serum and autologous IgG modified by treatment with a culture of a haemolytic streptococcus, *J. Med. Microbiol.* 5:1.
- Miller, K. B., and Schwartz, R. S., 1979, Familial abnormalities of suppressor-cell function in systemic lupus erythematosus, *N. Engl. J. Med.* 301:803.
- Min, K. W., Györkey, F., Györkey, P., Yium, J. L., and Eknayan, G., 1974, The morphogenesis of glomerular crescents in rapidly progressive glomerulonephritis, *Kidney Int.* 5:47.
- Morel-Maroger, L., Sraer, J. D., Herreman, G., and Godeau, P., 1972, Kidney in subacute endocarditis. Pathological and immunofluorescence findings, *Arch. Pathol.* 94:205.
- Morel-Maroger, L., Saimot, A. G., Sloper, J. C., Woodrow, D. F., Adam, C., Niang, I., and Payet, M., 1975, Tropical nephropathy and tropical extra-membranous glomerulonephritis of unknown aetiology in Senegal, *Br. Med. J.* 1:541.

- Morgan, A. G., and Soothill, J. F., 1975, The relationship between macrophage clearance of PVP and affinity of anti-protein antibody response in inbred mouse strains, *Nature* 254:711.
- Morita, T., Suzuki, Y., and Churg, J., 1973, Structure and development of the glomerular crescent, *Am. J. Pathol.* 72:349.
- Morley, A. R., Malick, S., McHugh, M., Quareshi, M., Ngu, J. L., and Wilkinson, R., 1982, Membranous glomerulonephritis associated with Loa loa: Demonstration of filarial antigens in membranous lesions, *N. Engl. J. Med.*, in press.
- Müller-Eberhard, H. J., 1968, The human complement system, in: *Textbook of Immunology* (P. A. Miescher and H. J. Müller-Eberhard, eds.), Grune & Stratton, New York, p. 33.
- Nagle, R. B., Ward, P. A., Lindsley, H. B., Sadun, E. H., Johnson, A. J., Berkaw, R. E. and Hildebrandt, P. K., 1974, Experimental infections with African trypanosomes. VI: Glomerulonephritis involving the alternate pathway of complement activation, *Am. J. Trop. Med. Hyg.* 23:15.
- Nash, T. E., 1974, Localization of schistosome antigen in *Schistosoma mansoni*, *Am. J. Trop. Med. Hyg.* 23:1085.
- Nash, T. E., Prescott, B., and Neva, F. A., 1974, The characteristic of a circulating antigen in schistosomiasis, *J. Immunol.* 112:1500.
- Natali, P. G., and Cioli, D., 1976, Immune complex nephritis in *Schistosoma mansoni* infected mice, *Eur. J. Immunol.* 6:359.
- Neva, F. A., Howard, W. A., Glew, R. H., Krotoski, W. A., Gam, A. A., Colins, W. E., Atkinson, J. P., and Frank, M. M., 1974, Relationship of serum complement levels to events of the malarial paroxysm, *J. Clin. Invest.* 54:451.
- Ngu, J. L., 1969, *The Nephrotic Syndrome in Adult Nigerians*, M.D. Thesis. University of Newcastle-upon-Tyne, Newcastle-upon-Tyne.
- Ngu, J. L., and Blackett, K., 1970, Complement and immunocglutinin changes in the nephrotic syndrome of adult Africans, *J. Trop. Med. Hyg.* 73:250.
- Ngu, J. L., and Blackett, K., 1976, Immunological studies in onchocerciasis in Cameroon, *Trop. Geogr. Med.* 28:111-120.
- Ngu, J. L., and Monekosso, G. L., 1968, Variation of serum complement levels in the nephrotic syndrome in Lagos, Nigeria, *J. Trop. Med. Hyg.* 71:252.
- Ngu, J. L., Adam, M., Leke, R., and Titanji, V., 1980, Proteinuria associated with diethylcarbamazine, *Lancet* 1:710.
- Ngu, J. L., Nduembe, P., Titanji, V., and Leke, R., 1981, A diagnostic skin test for *Onchocerca volvulus* infection. *Tropenmed. Parasitol.*, 32(3):170.
- Nishioka, K., and Linscott, W. D., 1963, Components of guinea pig complement. I. Separation of a serum fraction essential for immune hemolysis and immune adherence, *J. Exp. Med.* 118:767.
- Oldstone, M. B. A., 1975, Virus neutralization and virus-induced immune complex disease: Virus-antibody union resulting in immunoprotection or immunologic injury—two different sides of the same coin, in: *Progress in Medical Virology*, Volume 19 (J. L. Melnick, ed.), Karger, Basel, p. 84.
- Oldstone, M. B. A., and Dixon, F. J., 1969, Pathogenesis of chronic disease associated with persistent lymphocytic choriomeningitis viral infection: I. Relationship of antibody production to disease in neonatally infected mice, *J. Exp. Med.* 129:483.
- Oldstone, M. B. A., Tishon, J. M., Chiller, W. O., Weigle, W. O., and Dixon, F. J., 1973, Effect of chronic viral infection on the immune system: I. Comparison of the immune responsiveness of mice chronically infected with LCM virus with that of uninfected mice, *J. Immunol.* 110:1268.
- Oldstone, M. B. A., Theofilopoulos, A. N., Gunven, P., and Klein, G., 1974, Immune complexes associated with neoplasia: Presence of Epstein-Barr virus antigen-antibody complexes in Burkitt's lymphoma, *Intervirology* 4:292.

- Osawa, G., Beres, J., and Kimmelstiel, P., 1966, Glomerulonephritis: Observations by light and electron microscopy, *Am. J. Clin. Pathol.* 46:295.
- Ozawa, T., Levisohn, P., Orsini, E., and McIntosh, R. M., 1976, Acute immune complex disease associated with hepatitis: Etiopathogenic and immunopathologic studies of the renal lesion, *Arch. Pathol. Lab. Med.* 100:484.
- Paganelli, R., Ngu, J. L., and Levinsky, R. J., 1980, Circulating immune complexes in onchocerciasis, *Clin. Exp. Immunol.* 39:570.
- Passwell, J. H., Steward, M. W., and Soothill, J. F., 1974, The effects of protein malnutrition on macrophage function and the amount and affinity of antibody response, *Clin. Exp. Immunol.* 20:489.
- Petty, R. E., Steward, M. W., and Soothill, J. F., 1972, The heterogeneity of antibody affinity in inbred mice and its possible immunopathologic significance, *Clin. Exp. Immunol.* 12:231.
- Pillay, N. K., Kirch, E., and Kurtzman, N. A., 1973, Glomerulopathy associated with filarial loiasis, *J.A.M.A.* 225:179.
- Poon-King, T., Svartman, M., Mohammed, I., Potter, E. V., Achong, J., and Cox, R., 1973, Epidemic acute nephritis with reappearance of M-type 55 streptococci in Trinidad, *Lancet* 1:475.
- Prins, E. J. L., Hoorntje, S. J., Weening, J. J., and Donker, A. J. M., 1979, Nephrotic syndrome in a patient on captopril, *Lancet* 2:306.
- Randall, R. E., Abukurah, A. R., Tung, M. Y., Vaughan, E. R., and Still, W. J. S., 1971, Renal manifestations of chronic hepatitis, *J. Clin. Invest.* 50:75a.
- Ratnoff, O. D., and Naff, G. B., 1967, The conversion of C'Is to C'I esterase by plasmin and trypsin, *J. Exp. Med.* 125:337.
- Ree, G. H., 1976, Complement and malaria, *Ann. Trop. Med. Parasitol.* 70:247.
- Rickles, N., Zilberstein, Z., Kraus, S., Arad, G., Kaufstein, M., and Ginsburg, I., 1969, Persistence of group A streptococci labelled with fluorescein isothiocyanate in inflammatory sites in the heart and muscle of mice and rabbits, *Proc. Soc. Exp. Biol. Med.* 131:525.
- Sadigursky, M., Andrade, Z. A., Danner, R., Cheever, A. W., Kamel, I. A., and Elwi, A. M., 1976, Absence of schistosomal glomerulopathy in *Schistosoma haematobium* infection in man, *Trans. R. Soc. Trop. Med. Hyg.* 70:322.
- Sagnelli, E., Giuseppe, M., Guiseppa, P., Felaco, F. M., Filippini, P., Izzo, C. M., and Piccinino, F., 1980, Serum levels of hepatitis B surface and core antigens during immunosuppressive treatment of HBsAg-positive chronic active hepatitis, *Lancet* 2:395.
- Santoro, F., Borojevic, R., Bout, D., Tachon, P., Bina, J. C., and Capron, A., 1977, Mother-child relationship in human *schistosomiasis mansoni*: 1. Parasite antigens and antibodies in milk, *Am. J. Trop. Med. Hyg.* 26:1164.
- Seegal, B. C., Andres, G. A., Hsu, K. C., and Zabriskie, J. B., 1965, Studies on the pathogenesis of acute and progressive glomerulonephritis in man by immunofluorescein and immunoferritin techniques, *Fed. Proc.* 24:100.
- Shahin, B., Papadopoulou, Z. L., and Jenis, E. H., 1974, Congenital nephrotic syndrome associated with congenital toxoplasmosis, *J. Pediatr.* 85:366.
- Shulman, N. R., and Barker, L. F., 1969, Virus-like antigen, antibody and antigen-antibody complexes in hepatitis measured by complement fixation, *Science* 165:304.
- Shwe, T., 1971, Immune complexes in glomeruli of patients with leprosy, *Lepr. Rev.* 52:282.
- Sitprija, V., Pipatanagul, V., Boonpucknavig, V., and Boonpucknavig, S., 1974, Glomerulonephritis in typhoid fever, *Ann. Intern. Med.* 81:210.
- Slusarczyk, J., Michalak, T., Nazarewicz-de, M., Krawczynski, K., and Nowostawski, A., 1980, Membranous glomerulonephropathy associated with hepatitis B core antigen immune complexes in children, *Am. J. Pathol.* 98:29.
- Smith, M. D., Verroust, P. J., Morel-Maroger, L., Geniteau, M., and Coulaud, J. P., 1977, A study

- of the presence of circulating immune complexes in schistosomiasis, *Trans. R. Soc. Trop. Med. Hyg.* 71:343.
- Soothill, J. F., and Hendrickse, R. G., 1967, Some immunological studies of the nephrotic syndrome of Nigerian children, *Lancet* 2:629.
- Soothill, J. F., and Steward, M. W., 1971, The immunopathological significance of the heterogeneity of antibody affinity, *Clin. Exp. Immunol.* 9:193.
- Spitz, S., 1946, The pathology of acute *falciparum* malaria, *Milit. Surg.* 99:555.
- Srichaikul, T., Puwastein, P., Puwastein, P., Karnjanajetanee, J., and Bokisch, V. A., 1975, Complement changes and disseminated intravascular coagulation in *P. falciparum* malaria, *Lancet*. 1:770.
- Steward, M. W., 1977, Affinity of the antibody-antigen reaction and its biological significance, in: *Immunochemistry: An Advanced Textbook*, L. E. Glynn, and M. W. Steward, eds., John Wiley & Sons, London, 1977.
- Stokes, C. R., Swarbrick, E. T., and Soothill, J. F., 1980, Immune elimination and enhanced antibody responses; functions of circulating IgA, *Immunology* 80:455.
- Tabor, E., Aronson, D. L., and Gerety, R. J., 1980, Removal of hepatitis-B-virus infectivity from factor-IX complex by hepatitis-B immune globulin, *Lancet* 2:68.
- Tada, T., Kondo, Y. M., Okumura, K., Sano, M., and Yokogawa, M., 1975, *Schistosoma japonicum*: Immunopathology of nephritis in *Macaca fascicularis*, *Exp. Parasitol.* 38:291.
- Thompson, E. N., and Soothill, J. F., 1970, Chronic granulomatous disease: Quantitative Clinicopathological relationships, *Arch. Dis. Child.* 45:24.
- Treser, G., Semar, M., Ty, A., Segal, I., Franklin, M. A., and Lange, K., 1970, Partial characterization of antigenic streptococcal plasma membrane components in acute glomerulonephritis, *J. Clin. Invest.* 49:762.
- Tu, W. H., Shearn, M. A., and Lee, J. C., 1969, Acute diffuse glomerulonephritis in acute staphylococcal endocarditis, *Ann. Intern. Med.* 71:335.
- Turner, M. W., 1982, *Complement in Paediatric Immunology* (J. F. Soothill, A. R. Hayward, and C. B. S. Wood, eds.), Blackwell, Oxford, in press.
- Unanue, E. R., Dixon, F. J., and Feldman, J. D., 1967, Experimental allergic glomerulonephritis induced in the rabbit with homologous renal antigens, *J. Exp. Med.* 125:163.
- Vallota, E. H., and Müller-Eberhard, H. J., 1973, Formation of C3a and C5a anaphylatoxins in whole human serum after inhibition of the anaphylatoxin inactivator, *J. Exp. Med.* 137:1109.
- Van Marck, E., Deedler, A. M., and Gigase, P. L. J., 1977, Effect of partial portal vein ligation on immune glomerular deposits in *Schistosoma mansoni* infected mice, *Br. J. Exp. Pathol.* 58:412.
- Vassalli, O., and McCluskey, R. T., 1971, The pathogenetic role of the coagulation process in glomerular diseases of immunological origin, *Adv. Nephrol.* 1:47.
- Verroust, P. J., Wilson, C. B., Cooper, N. R., Egington, T. S., and Dixon, F. J., 1974, Glomerular complement components in human glomerulonephritis, *J. Clin. Invest.* 53:77.
- Verroust, P. J., Adam, C., Smith, M. D., Richard-Lenoble, D., Kourilsky, O., and Morel-Maroger, L., 1979, Circulating immune complexes and C3d in human parasitosis, *Kidney Int.* 16:19.
- von Lichtenberg, F., Sadun, E. H., and Bruce, J. I., 1972, Renal lesion in *Schistosoma japonicum* infected rabbits, *Trans. R. Soc. Trop. Med. Hyg.* 66:505.
- von Schéele, C., Althoff, P., Kempf, V., and Schelin, U., 1976, Nephrotic syndrome due to subacute glomerulonephritis—association with hydrocarbon exposure? *Acta. Med. Scand.* 200:427.
- Waksman, B. H., and Cook, J. A., 1975, A report of a conference on newer immunologic approaches to schistosomiasis, *Am. J. Trop. Med. Hyg.* 24:1037.
- Wannamaker, L. W., 1970, Differences between streptococcal infections of the throat and of the skin, *N. Engl. J. Med.* 282:23.
- Ward, P. A., 1974, Leukotaxis and leukotactic disorders: A review, *Amer. J. Pathol.* 77:520.

- Ward, P. A., and Kibukamusoke, J. W., 1969, Evidence of soluble immune complexes in the pathogenesis of the glomerulonephritis in quartan malaria, *Lancet* 1:283.
- Weigle, W. O., 1961, Fate and biological action of antigen-antibody complexes, in: *Advances in Immunology*, (W. H. Taliaferro and J. H. Humphrey, eds.), Academic Press, New York, pp. 282-316.
- Wheeler, J., Holland, J., Menzies, S., and Blainey, J. D., 1975, Immunologic response to glomerular basement membrane and streptococcal antigens in immunized rabbits, *Immunology* 28:653.
- Whittle, H. C., Abdullahi, M. T., Fakunie, F., Parry, E. H. O., and Rajkovic, A., 1973, Scabies pyoderma and nephritis in Zaria, Nigeria, *Trans. R. Soc. Trop. Med. Hyg.* 67:349.
- Wickbom, B., and Winberg, J., 1972, Coincidence of congenital toxoplasmosis and acute nephritis with nephrotic syndrome, *Acta Paediatr. Scand.* 61:470.
- Wilens, S. L., and Henderson, D., 1965, Glomerulonephritis in pressor drug-enhanced serum sickness of rabbits, *Proc. Soc. Exp. Biol. Med.* 120:866.
- Williams, A. I. O., and Houba, V., 1972, Soluble serum antigens of *P. falciparum* in Nigerians: I. Local incidence of malarial soluble serum antigens and antibodies, *Afr. J. Med. Sci.* 3:295.
- Wilson, C. B., and Dixon F. J., 1976, The renal response to immunological injury, in: *The Kidney* (B. M. Brenner, and F. C. Rector, eds.), W. B. Saunders, Philadelphia p. 870.
- Wilson, R. J. M., 1970, Antigen and antibodies associated with *Plasmodium falciparum* infections in West Africa, *Trans R. Soc. Trop. Med. Hyg.* 64:547.
- Wilson, R. J. M., McGregor, I. A., Hall, P., Williams, K., and Bartholomew, R., 1969, Antigens associated with *Plasmodium falciparum* infections in man, *Lancet* 1:201.
- Wilson, R. J. M., McGregor, I. A., and Williams, K., 1975a, Occurrence of S-antigens in serum of *Plasmodium falciparum* infections in man, *Trans. R. Soc. Trop. Med. Hyg.* 69:453.
- Wilson, R. J. M., McGregor, I. A., and Hall, P. J., 1975b, Persistence and reoccurrence of S-antigens in *Plasmodium falciparum* infections in man, *Trans. R. Soc. Trop. Med. Hyg.* 69:460.
- World Health Organization, 1977, The role of immune complexes in disease. *WHO Tech. Rep. Ser.* 606.
- Yoshizawa, N., Treser, G., Sagel, I., Ty, A., Ahmed, U., and Lange, K., 1973, Demonstration of antigenic sties in glomeruli of patients with acute poststreptococcal glomerulonephritis by immunofluorescein and immunoferritin techniques, *Am. J. Pathol.* 70:131.
- Zabriskie, J. B., 1971, The role of streptococci in human glomerulonephritis, *J. Exp. Med.* 134:180.
- Zimmerman, S. W. Groehler, K., and Beirne, G. J., 1975, Hydrocarbon exposure and chronic glomerulonephritis, *Lancet* 2:199.
- Zuidema, P. J., 1971, Renal changes in loiasis, *Folia Med. Neerl.* 14:168.

Nutrition and Work Performance

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1. INTRODUCTION

Nutritionists have focused their special attention on work performance and fitness for many reasons during the last two decades. We can mention here some efforts to insure desirable growth and development not only from the point of view of body size but also from the point of view of the functional capacity, care for health, and its prognosis in later periods of life, as well as an active life. Further, there exists also the problem of economic productivity, an important concern both for the individual and for the national economy.

These topics, especially working efficiency, were discussed in the FAO Basic Study in 1962. The impact of poor diet was found to be an important cause of reduced work capacity for the following reasons:

1. The body tends to protect itself against the lack of proper food by avoiding effort, the final results being lethargy and lack of initiative and drive.
2. Poor diet lowers the worker's resistance to disease.
3. It leads to a considerable amount of absenteeism because of sickness.
4. Where workers are undernourished and tend to tire quickly, accident rates tend to be higher.

Malnutrition that exceeds the adaptation limits of the organism, especially during growth, has deteriorating effects on the development of the human organism during the whole life span. The susceptibility to infections and infestations is

markedly increased by malnutrition with a resulting increased morbidity and mortality during early periods of life. The delayed effects in the case of survival are manifested with regard to body size, functional capacity, and health (McCance, 1963).

But it is necessary to keep in mind that there exist different types of malnutrition and that it is necessary to specify as exactly as possible their character, duration, degree, time of onset during ontogeny, etc. This is a very difficult task; certain confusion that often originates from the comparison of the consequences of malnutrition in various studies is obviously the result of the fact that these differences and variations are not always considered and evaluated.

Special nutritional situations result from the general, overall restriction of all nutrients and energy of varying degrees, or from protein–energy malnutrition, or from energy malnutrition combined with special lack of vitamins, minerals, etc. Another case is iron-deficiency anemia, mostly combined with general malnutrition.

Moreover, the situation is not the same when any of these restrictions begins during the fetal, weaning, adolescent, or adult period of life. An organism born from a mother malnourished during her whole life prior to pregnancy and also malnourished during lactation will probably differ from an organism malnourished later during ontogeny. This will surely be reflected not only in body size, health, and its prognosis but also in the functional capacity and work performance of the organism. Another special situation is the status of acute starvation or semistarvation in the period when the organism has reached its maturity.

A number of clinical and epidemiologic observations have initiated experimental studies in humans as well as in laboratory animals. The latter can help in the solution of more basic physiological problems concerning the impact of malnutrition on the development as well as functional capacity of selected organs and/or systems most closely related to physical performance. It will be useful to mention some of these studies in this survey. Nevertheless, they are not sufficient to completely answer the question of physical performance in human subjects on the basis of experiments on laboratory animals differing especially from the point of view of higher nervous activity, psychological traits, motivation, etc.

Last but not least, physical performance and functional capacity have many differentiated aspects which might not be influenced by the impact of malnutrition in the same manner, degree, timing, etc. Factors constituting physical performance can be defined according to the classical *Textbook of Work Physiology* by Åstrand and Rodahl (1977) and many others as follows: (1) energy output consisting of both aerobic and anaerobic processes, (2) neuromuscular function including strength and technique, and, finally, (3) psychological factors of motivation and tactics (having in mind time schedule, grading, rest intervals, etc. regarding special physical performances). If we keep in mind the above-men-

tioned malnutrition situations and their impact from the point of view of various items of physical performance, a much more complicated picture arises.

Increased attention to problems of functional capacity and work performance are fully justified: *inter alia*, a good level of fitness means a positive aspect of human health (i.e., not merely the absence of an illness) and also a better prognosis for longevity, longer survival, and full economic productivity both from the mental and physical point of view.

In conjunction with the abovementioned aspects, it is necessary to emphasize that the status of malnutrition does not refer only to quantitative and/or qualitative restriction of diet but also to the opposite situation, i.e., the status of quantitative and qualitative hyperalimentation so common in the industrially developed countries and sometimes also in the developing countries. A too abundant and unbalanced diet, as related to the needs of the organism, is also a risk as regards functional capacity, work performance, health status, longevity, and survival. With regard to physical performance, we must try to define an optimum diet in terms of the economic possibilities of the present world as well. Obviously, the actual status as regards nutrition not only in developing but also in the industrially developed countries does not set an example that ought to be followed exactly and fully in the future. Morbidity and mortality data, especially on "diseases of civilization" in the industrially developed countries, show this very clearly. We must not forget either the economic price and all circumstances of this status, i.e., per capita price of food consumed per year.

With the achievement of an optimal work performance and functional capacity in mind, it is necessary to define suitable individually differentiated diets from the quantitative, qualitative, and also economic point of view, considering the availability and price of individual foodstuffs in various countries. Only then can the maximum number of people be satisfied.

2. INDIVIDUAL ASPECTS OF PHYSICAL PERFORMANCE

It is generally known that the capacity of an organism to accomplish physical work diminishes with increasing energy malnutrition. Weakness is one of the common characteristics of marked energy restriction. In extreme cases, there is not enough strength and endurance to walk or even to stand. The individual is prostrate, quite passive physically and mentally. The progress of such changes depends on the degree of the energy restriction and on further complicating factors (special deficiencies, simultaneous infections, etc).

Physiological studies of nutritional status in humans cannot be limited to resting or basal conditions, because the functional capacity of an organ and system can be evaluated only when subjected to functional loads.

The relationships between nutrition and performance and/or working efficiency have been reviewed by many authors such as Loewenstein (1968), Keller and Kraut (1962), Bhavani and Delavadi (1966), and others.

An adequate somatic development and body size are assumed to be prerequisites of good physical performance. Optimal absolute and relative body height and weight, weight for age, and weight for height are evaluated, as compared to national standards. In addition, various circumferential measurements (e.g., chest, arm) are used for the evaluation of the past and actual nutritional status.

Body composition, e.g., the absolute and relative amounts of lean body mass and depot fat using various methods [^{40}K measurements, densitometry, hydrometry, etc.; see surveys of Keys and Brožek (1953), Moore *et al.*, 1963], serves as an indicator of the adequacy of somatic and nutritional status (Stini, 1981). For measurements under field conditions, skin folds measured by different calipers are used. Triceps and subscapular skin folds are measured most often.

Aerobic and anaerobic capacity for physical performance depend on the levels of aerobic and anaerobic processes. The physiology of muscular work is basically the chemistry and physics of the transformation of chemically bound energy from food and bodily reserves into mechanical energy. The energy for the performance of physical work is ultimately derived from the food eaten. Chemical energy is transformed into kinetic energy and heat, which is possible only in the presence of oxygen; its function is aerobic. The common means for the transfer of energy within the living organism are high-energy phosphate compounds.

The oxygen consumption is markedly increased during muscular work, thus requiring an increased effort from the cardiorespiratory system. As a measure of the aerobic capacity or oxygen ceiling, the evaluation of the oxygen uptake during a maximal work load on a treadmill or a bicycle ergometer is most often used. The two methods mentioned characterize the efficiency of both circulatory and respiratory systems which insure the transfer of oxygen to the working tissues, mainly skeletal muscles. For the evaluation of the cardiorespiratory system and aerobic power, a number of further tests are used, e.g., the step test in various modifications (see Åstrand and Rodahl, 1977) or other forms of physical loading (Ström, 1962).

For the testing of anaerobic power, the subject is suggested (Weiner and Laurie, 1969) to run up a staircase at top speed; horizontal and vertical dimensions of the step, time of taking off for the fourth and sixth jump, and body weight are evaluated. In laboratory testing, the oxygen debt, lactate concentration, and further parameters are used. The relationship between aerobic and anaerobic powers is also of basic importance for a good level of work performance (Yoshimura and Weiner, 1966; Åstrand and Rodahl, 1977).

Lung capacity is measured as the forced expiratory volume and the forced vital capacity.

Among neuromuscular functions, muscle strength is mostly tested by different dynamometers, electrical and mechanical, adapted for the evaluation of various muscle groups. These procedures are described in the above-mentioned textbooks and handbooks. Electromyography serves the purpose of more detailed analysis of the electrical activity of skeletal muscles during work load.

Coordination of more complex movements, when numerous muscle groups are active, is conditioned by the timing of the period of muscular tension development in a movement cycle. Various tests exist and are also widely used for nutrition research.

Muscular fatigue after a work load develops much sooner in malnourished subjects than in well-nourished ones. Such fatigue is of both central and peripheral origin and limits the working capacity in the malnourished.

Technique can be evaluated by a number of tests—e.g., pull-ups, sit-ups, shuttle running, standing broad jumps, 50-m dashes, softball throw distance, 600-yd run—walk—as recommended by the American Association for Health, Physical Education and Recreation, International Biological Program methodology (Weiner and Laurie, 1969). All these tests characterize coordination and technique, as well as other aspects of physical performance—speed, endurance, strength of selected muscle groups, and so forth. The battery of such tests is great and varies according to different authors in fitness research.

Motivation and tactics also belong among necessary components of good physical performance. Their status and degree can be evaluated according to the results of special physical tasks when comparing different groups, by psychological tests, etc.

3. THE IMPACT OF MALNUTRITION

3.1. Studies in Laboratory Animals

Body size can be markedly influenced by maternal dietary restriction during pregnancy: maternal weight gain and fetal birth weight in the rat are reduced (Anderson *et al.*, 1980). Raising or lowering the standard of nutrition during the suckling period can change the body size and weight of adult rats; however, changes in nutrition later on have much smaller effect. This does not necessarily mean that energy restriction in early postnatal life or even *in utero* always results in a reduced body size in adulthood. But in some experiments (McCance, 1963), cockerels and pigs had a reduced final stature as a result of profound undernutrition for long periods of time after birth. Undernutrition from the third to the 12th

weeks of life in rats caused reduced growth which persisted even after a longer period of *ad libitum* feeding (Widdowson, 1964). Along with growth failure of malnourished rats, the growth hormone blood level is lower (Nitzam and Wilber, 1974).

Protein–energy malnutrition during the weaning period of rats with growth failure causes the suppression of longitudinal bone growth; bone width relative to length is also altered. During the period of undernutrition, the calcified tissues are particularly affected. The bones become abnormal in structure, and the cortex of the long bones is also abnormal in composition (Pratt and McCance, 1964). Another experiment showed small, poorly remodeled long bones in undernourished pigs. These bones contained little trabecular and cortical bone. The epiphyses were in an advanced state of development for the length of bone (Adams, 1969).

Lee and Mayers (1979) showed that during protein–energy malnutrition appositional bone growth in the seventh caudal vertebra of the rat was slowed and finally ceased. Appositional growth began again and attained an excess rate during realimentation which may account for changes in the skeletal proportions of the malnourished. Protein- and energy-malnourished monkeys showed an emaciated appearance often associated with marasmus. Redistribution of tissues appeared over a slowly expanding skeleton. For many skeletal proportions, the pattern and shape differed (Fleagle *et al.*, 1975). Maternal malnutrition during pregnancy also delays the appearance of the ossification centers in the embryo (Shrader and Zeman, 1973) and later. Bone growth and maturation in protein–energy malnutrition from the point of view of histology, composition, weight, density, etc. were reviewed by Himes (1978): it is clear that bone development is affected by protein–energy malnutrition both before and after birth; it is not clear, however, whether these bone responses are beneficial adaptations or not.

Skeletal muscle mass is decisive for physical performance as is growth in height. It is also the largest endogenous source of amino acids. Therefore, in the case of starvation or malnutrition, this reserve can be mobilized to satisfy amino acid requirements and, to a lesser extent, energy requirements (Cahill, 1970). Protein malnutrition and/or protein–energy malnutrition cause a substantial loss of skeletal muscle mass: in adult animals, this does not involve changes in muscle fibers but, rather, the removal of myofibrillar material from previously existing units. There is a loss of protein from the contractile tissue but not from the connective tissue and collagen. It takes about 1 week before muscles begin to lose greater amounts of weight; nevertheless, changes in protein metabolism appear sooner. Low and Cerauskis (1977) showed that the synthesis of sarcoplasmic and myofibrillar proteins was equally affected, i.e., it decreased during 48 hr of fasting in rat cardiac, diaphragm, and leg muscles. A different situation might be found in growing animals.

There exist contradicting findings on the effect of food restriction on the development of the number of muscle fibers. A decrease in diameter was reported, but no change in the number of fibers was found when muscle weight of mice was reduced by severe food restriction. Some authors showed reduction in both fiber number and fiber diameter in muscle of rats deprived of all food, but others found only a reduction in fiber diameter of various muscles in young undernourished rats (for citations, see Hegerty and Kim, 1980) as well as pigs (Stickland *et al.*, 1975). A reduction of both number and diameter of the muscle fibers in m. plantaris, soleus, and biceps brachii was found after the withdrawal of all food until 40% weight loss in weanling rats (Hegerty and Kim, 1980). This change was not of the same degree in individual muscles. It was also speculated that fiber fusion occurred. Refeeding the rats to their original body weights eliminated the decrease in either the number and diameter of muscle fibers or both. It seems that skeletal muscle in rats has a good capacity for recovery in a short time from extensive weight loss when the normal body weight is attained. But as mentioned above, the experiments did not have the same setup; there were differences in animal species used, the type and duration of malnutrition, the age of its onset, as well as the age when the resulting changes in the muscle were studied.

There is some evidence that early malnutrition, i.e., from birth up to 13 weeks, in rats causes functional changes manifested even after rehabilitation: both *in vitro* and *in vivo* studies in the gastrocnemius muscle showed earlier fatigability after direct electrical stimulation. An increase in glycolytic activity and glycolytic enzymes and a decrease in the oxidative capacity were also observed (Raju, 1974).

Acute starvation or malnutrition influences muscle metabolism. Thus, the incorporation of [^{14}C]phenylalanine by skeletal muscle ribosomes from rats given a protein-free diet or starved for 2 days was decreased. In rats refed after starvation for 2 days, muscle protein synthesis recovered more efficiently in those given a protein-free diet. Muscle protein synthesis recovered after rats were refed with different diets (Nakono, 1978).

Experiments were also done on marginally malnourished (Stewart, 1976) and normally fed rats kept for 11 generations. During normal growth and development, there was a decrease in the RNA : protein ratio in both colonies, similar to the reported developmental changes in the rate of protein synthesis. The cause of failure of catch-up growth of the marginally malnourished rats was not a decrease in RNA concentration in the gastrocnemius muscle but reduced nuclear proliferation and protein accumulation, leading to hyperplastic and hypertrophic growth failures in most stages (Nnanyelungo, 1978).

Less is known about the effects of malnutrition on cardiac muscle. There is some evidence that similar metabolic changes occur, but they are of less magnitude (Widdowson, 1964, 1969). Acute protein malnutrition has an adverse effect

on myocardial function in adult (400-g) rats given an isocaloric diet without protein for 2 days. The stroke volume was significantly lower in protein-depleted rats when left atrial pressure exceeded 10 cm H₂O. Cardiac output and aortic flow were significantly lower when left atrial pressure exceeded 25 cm H₂O. Coronary flow and heart rate did not differ significantly, although the protein-depleted group did not match the increased heart rate developed at higher left atrial pressure by the control group (Kyger *et al.*, 1978).

Experiments of Widdowson and McCance (1956) revealed that in young and adult rats the consequences of chronic and/or normal nutrition followed by starvation were similar. Age and sex sometimes made a difference, e.g., in the amount of nitrogen in the liver and the bodies, hemoglobin concentration, fat and calcium content in the organism.

Undernutrition in one generation affected the cellular development in different organs in a similar fashion in the next generation, but when the results for composition of the progeny were expressed per 100 g body weight, many of the changes disappeared (Srivastava *et al.*, 1974).

Postnatal undernutrition during the period of proliferative brain cell growth will retard the rate of cell division regardless of the region or cell type and results in a permanent reduction of brain cell number. Prenatal malnutrition in the rat will do precisely the same thing, and combined prenatal and postnatal undernutrition will act synergically, producing a profound reduction of brain cell number (Winick, 1970). The electrocorticogram in the malnourished rats is altered—there is a higher proportion of slow waves and a lower voltage of the electrocorticogram up to the 16th day, when the differences disappear (Salas and Cintra, 1975).

Special deficiencies such as iron deficiency significantly decrease the voluntary activity of rats when hemoglobin dropped to less than about 11 g/100 ml. After 3 min of exercise, the heart rates were significantly higher. After an exhaustive run, blood lactate was higher in anemic rats, and mean running time was significantly decreased. Twitch tension (7 Hz) of the gastrocnemius muscle *in situ* was 50% and 30% of resting tension after 5 min of stimulation in control and anemic rats, respectively. Obviously, exercise is a significantly greater cardiovascular and metabolic stress on anemic than control rats (Edgerton *et al.*, 1977).

Moderate iron deficiency anemia did not stimulate changes of the oxidative and glycolytic capacity of skeletal muscle, whereas severe iron deficiency anemia induced rapid changes in the mitochondrial component of skeletal muscles. Hemograms in conjunction with muscle biochemistry in the moderately anemic group suggest hypochromic, microcytic, manifest iron deficiency anemia, whereas those of the severely anemic group suggest hypochromic, normocytic “severe iron deficiency anaemia” (Koziol *et al.*, 1978).

Selected examples show marked influence of malnutrition, protein–energy

malnutrition, and such special deficiencies as iron deficiency on general and tissue growth and metabolic processes in the organism and tissues. These effects can finally be reflected in the functional capacity, voluntary activity, and fitness of the organism at the present time or later in life.

These changes depend on the degree of malnutrition and age. Marginal malnutrition can have a certain impact but, on the other hand, can manifest itself positively in certain aspects. Permanent reduction of the food intake throughout life to 80 or 60%, respectively, led to the lowest mortality of rats as compared to *ad-libitum*-fed controls (Nolen, 1972).

Protein–energy malnutrition during lactation and then up to the 49th day of life of rats resulted during realimentation in a significantly increased level of voluntary activity in rotation wheels (only until adult age; Figure 1), changes in selected parameters of lipid metabolism (Pařízková and Petrásek, 1979), lower energy cost of weight increments during certain periods of growth (Pařízková *et al.*, 1979), and, finally, in a lower sensitivity of the cardiac muscle to isoproterenol which caused less cardiac damage as compared to controls fed during their whole lives *ad libitum* and without possibility of exercise. Body size and weight remained permanently lower, but generally, the late consequences of the early malnutrition were beneficial when one considers the sensitivity of heart muscle to this noxious agent (Pařízková, 1978a,b,c, 1979, 1981; Pařízková *et al.*, 1981).

Clearly, it is necessary to specify exactly the type of malnutrition, its degree and character: the consequences can vary markedly, and they are not always entirely negative. When the restriction of energy intake is within the adaptation limits of the organism, some positive manifestations such as greater resistance of the heart or longer survival may appear.

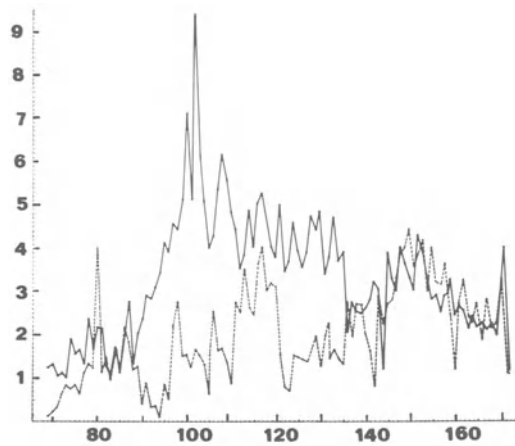


Figure 1. Spontaneous motor activity in rotation wheels in male rats with protein–energy malnutrition at the beginning of life (PEM, full line, $n = 11$) and controls (C, interrupted line, $n = 8$). Abscissa, days of life; ordinate, thousands of meters run per day.

3.2. Human Studies

3.2.1. Experimental Models

Available data show that the impact of short energy restriction (10 days, 420 kcal per day) does not cause much deterioration of the level of functional capacity: parallel to weight reduction, oxygen consumption during standardized sub-maximal and maximal exercise on a treadmill was not significantly influenced (Daws *et al.*, 1972). After a maximal work load, the recovery pulse rates were significantly reduced as the study progressed, showing very rapid recovery of the heart and increased efficiency. Energy restriction did not produce a significant decrease in the stamina test. This study indicates that the described type of work does not appear to be impaired by short temporary energy restriction.

The situation is different in the case of energy and water deprivation (3 days) in young men tested from the point of view of muscle strength and endurance which both decreased (Bosco *et al.*, 1974).

Semistarvation in normally nourished adult men was thoroughly studied by Keys *et al.* (1950) in the Minnesota experiment. In 6 months of semistarvation, young male volunteers lost 24% of their body weight, most of which was the loss of muscle mass, including the heart (caused by shrinking of individual muscle fibres), and of body fat.

3.2.1a. Respiratory System. As regards respiration in the semistarved subjects, there was a tendency toward a decreased rate and decreased tidal volume, which finally resulted in a slight but definite average diminution in the respiratory volume. It was concluded on the basis of previous experiments (Carnegie experiment) that a reduced diet produced a decrease in the sensitivity of the respiratory center.

In the Minnesota experiment, the vital capacity was definitely and progressively diminished, and this change was reversed by realimentation. The pulmonary ventilation during basal rest, measured as the total respiratory volume per minute, decreased by 30.6%. The measurements of ventilation of the lungs during aerobic work (walking on a treadmill for 30 min) showed a marked reduction; the respiratory efficiency (expressed as cubic centimeters of oxygen used per liter of expired air) decreased by 16.2%. In the early stage of realimentation, the ventilation in aerobic work tended to decrease further, even though the body weight was rising, and therefore the physical work increased. Later during rehabilitation, ventilation rose sharply and surpassed the control level (Keys *et al.*, 1950).

The respiratory efficiency in basal rest declined during semistarvation by 11%.

3.2.1b. Cardiovascular System. Among symptoms of severe undernutrition belong bradycardia, hypotension, lowering of the skin temperature, and

often vertigo and slight cyanosis, which are all related to the heart and circulation. Rates as low as 30 to 40 beats per min were often observed during bed rest; the minimum rate found was 26 beats per min (observed during World War II, in a Warsaw Ghetto,). Irregularities of the rate were not observed (Keys *et al.*, 1950).

Slight to moderate hypotension was found in uncomplicated severe malnutrition: systolic pressure of 110 to 130 mm, sometimes 90 mm or lower. Diastolic pressure fell to approximately 70 to 80 mm but could even be 55 mm. The decline was greatest in those groups in whom blood pressure was highest before the famine.

The heart sounds were sometimes described as "soft and muffled." On the other hand, the heart sounds were often normal or very clear and loud, no doubt because of the thinness of the chest wall (see Keys *et al.*, 1950).

The complaint of feeling of coldness is very common among undernourished people. The skin is cold to the touch, which is attributed to the general reduction of body temperature which is often observed (down to 35°C or below). The suggestion that the peripheral circulation is diminished is also supported by the pallor of the skin, which is out of proportion to the slight anemia which is usually present, and by the fairly frequent appearance of slight cyanosis without dyspnea. There are few clinical studies on the effect of energy restriction on hypertensive heart disease although the possibility of an important effect seems clear.

The Minnesota experiment confirmed further findings on the impact of semistarvation: pulse rate and systolic blood pressure declined. The decrease of the diastolic blood pressure was relatively smaller than that of the systolic. Cardiac output diminished as shown by roentgenokymographic volume measurements. The stroke volume decreased by 18.1% on the average.

The work of the heart in the Minnesota experiment (function of the amount of blood pumped, the arterial blood pressure, and the velocity imparted) was reduced to half of the control values after 6 months of semistarvation. Oxygen pulse (cubic centimeters of oxygen used per minute divided by the heart rate per minute) was reduced from the initial values of 4.20 to 3.84.

Most observations confirm that the heart is not specifically affected in starvation and that the depression of the circulation is not serious in view of the reduction in the metabolic demand on the circulation. Tachycardia, irregularities of the rhythm, dyspnea, precordial pain, elevated venous pressure, enlargement of the heart, claudication, and marked cyanosis are not present. Nevertheless, some inadequacies of the circulation might contribute to edema. Some of the circulatory changes can be explained as protective phenomena preserving the strength of the heart. The prolongation of the systole also suggests preservation of energy. The decreased irritability of the heart also affords protection. But generally, in starvation, the heart is not normal and obviously does not maintain its circulatory function even in the proportion to the reduced metabolic needs.

Roentgenokymographic measurement of the heart showed a decrease in all dimensions of the heart during semistarvation and a more upright position in the chest. The total volume reduction of the heart averaged 16%. The mean oxygen saturation of the venous blood fell from 20 to 30%, and the oxygen content from 35 to 40% (Keys *et al.*, 1947). More recently, nutritional microcardia was observed, e.g., in selected malnourished African subjects who had sprue. After treatment and realimentation, the consequences of malnutrition were reversed (Gelfland *et al.*, 1975).

3.2.1c. Neuromuscular Function and Motor Performance. Gross morphology shows that the brain and nervous system are relatively less influenced by semistarvation than are other tissues (although the situation is different during growth). Even microscopic examinations failed to reveal more marked abnormalities when changes resulting from starvation must be assumed. Paresthesia, e.g., numbness, tingling, burning, were noted frequently at the tips of fingers and toes in semistarved prisoners evacuated from Germany in 1945. The results of functional examinations of the nervous system were largely negative in semistarved patients who lost 20 to 40 kg of body weight and developed edema. Only the patellar reflexes were affected. Muscular pain, weakness and paresthesias in the limbs were observed in another experiment; the prisoners were forced to reduce their work output, and some had to quit completely, after their energy input was decreased from the initial 3400 to 1700 kcel (14.22 to 7.11 MJ).

In the Minnesota experiment, the tendon reflexes (the patellar and the Achilles) were diminished in the majority of men. Motor performance was altered in this experiment similarly to what has been recorded in field reports from areas of famines and semistarvation. Decrease of muscular endurance and strength, the deterioration of coordination, and the slowing down of voluntary movements were observed.

Hand grip strength measured by hand dynamometer diminished after 24 weeks of semistarvation by 28.2%. The strength of the back (back lift dynamometer) was reduced by 29.8%. Gross body reactions (time in 0.01 sec) increased by 6.0%. Manual speed (evaluated as the number of balls per minute) decreased by 5.2%. Tapping was slower (taps per 10 sec) by 6.4%. Pattern tracing (i.e., number of error contacts in 0.25 sec) increased by 16.3%.

The largest deterioration was obtained in tests in which peripheral factors such as the state of the muscles limited the performance capacity. Tests in which the central nervous system was the critical component, such as the test of speed of tapping, displayed changes that were consistent from subject to subject and therefore statistically significant but of little biological importance. Therefore, it is evident that, under conditions of famine, the capacity for work will be most seriously affected in jobs requiring lifting, pushing, and carrying of loads. The ability to climb, to walk long distances, or to stand for prolonged periods of time will also be diminished. Performances involving speed and accuracy may be

expected to be less impaired. But these conclusion were based on tests of short duration (Keys *et al.*, 1950). Moreover, the situation might be different in subjects who adapted to reduced energy intake not suddenly, following normal diet, but for longer periods of time, for instance, from earliest childhood. Ethiopian long-distance runners, who have a not too abundant food intake as suggested by low values of height and weight, have had repeatedly amazing performances at the Olympic Games, much better than those of their more nourished and larger peers.

Work performance in aerobic work deteriorated after semistarvation. The total oxygen consumption during standard work of moderate intensity decreased by 28.3%, and there was no net change in the energy cost of work per kilogram body weight. There developed a relative tachycardia; the oxygen pulse decreased by 25.8%. The average fitness score (Harvard Fitness Test) had decreased to 52% of the control value, and the recovery of strenuous work capacity was surprisingly slow during rehabilitation.

Total aerobic capacity, measured as oxygen uptake during a maximal work load on a treadmill, deteriorated too: the maximal oxygen uptake (ml/kg body weight) was reduced by 25% (Keys *et al.*, 1950).

3.2.2. Nutritional Status and Work Performance in Developing Countries

Extensive data on work performance were assembled in various populations with different nutrition by studies under the International Biological Programme (IBP) (Chakraborty and Guha Roy, 1966). These and other studies were, however, mostly performed without simultaneous evaluation of energy intake and nutritional status (Maitra *et al.*, 1966; Elsner, 1966; Lahiri, 1977; Sirotnin and Matsynin, 1977), which can be, nevertheless, in certain cases ascertained from the height : weight ratio, or body composition. Diet was also studied separately (Roy, 1966; Boyo, 1977). Only in selected cases did these studies include nutrition surveys (Norgan *et al.*, 1974; Norgan and Ferro-Luzzi, 1978).

The responses to work load of different ethnic groups have been studied and compared. Anderson (1973) found reduced values of aerobic capacity in Ethiopian men: nutrition was not checked, but very low values of absolute and relative weight indicate malnutrition. Hemoglobin levels of the Ethiopians were 85% of the European values. Physiological responses to a work load and aerobic capacity in tribal African men of the Central Congo (Ghesquierre and Anderson, 1973) who had higher body weights were nevertheless quite similar to the standard European men of similar size or to Kalahari Bushman and Bantu men in South Africa (Wyndham *et al.*, 1966; Wyndham, 1977). Adequate body size as a characteristic of proper somatic development and satisfactory nutritional status seems to be very important for performance and energy expenditure.

Complex studies were performed in New Guinea by Hornabrook (1977)

who followed, *inter alia*, physique, health, nutrition, and energy expenditure together with fitness and climatic adaptation. He found no signs of clinical malnutrition, with the energy intake 8.12 to 10.55 MJ for men, and 5.95 to 8.81 MJ for women in two localities—coastal and highland. Intakes of protein were low, ranging from 6.0 to 7.2% of the energy value of diets; fat provided only 10 to 17%. Ventilatory capacity in healthy highland New Guineans was similar to that for Europeans; coastal people had lower values. Total lung capacity and the transfer factor for adult highlanders were larger than for coastal people. Asahina (1977) followed people in various parts of Japan with regard to work capacity and nutrition. According to his data, an increased protein intake improved work capacity, energy metabolism, and development of bones and muscles. Baker (1977) studied the aerobic capacity in Andean Quechua Indians together with nutrition. Maximum oxygen uptake was higher than, e.g., that in United States males (49.1 ml compared to 38.1 ml/kg body weight per min). Surveys of food intake generally showed dietary balance, with possible exceptions for pregnant women and children.

Barbashova (1977) followed indigenous and nonindigenous residents of Nenets National Area of the Archangel region in the USSR. She found the resting metabolic rate to be 10 to 15% higher than the European standards, and the pulmonary ventilation was increased. Bradycardia was proved in addition; peripheral blood flow and limb skin temperature were found to be higher than normal and might contribute to a greater tolerance to cold. Nutrition was found to be satisfactory; total energy intake during physical performance was increased by 400–500 kcal, i.e., 1.67–2.09 MJ. Relationships between individual main nutrients were satisfactory, but hypovitaminosis of C, B₂, B₆, PP, A, and D was observed almost everywhere in the circumpolar regions. Low working capacity, fatigue, inertia, and depression were more frequent during the polar night. Symptoms of increased psychic activity, nervousness, impatience, affection, and hysterical reactions predominated during the polar day.

Daily work productivity is simultaneously related to the aerobic capacity, as expressed in values of the maximal oxygen uptake ($V_{\max}$), height and body fat ($r = 0.685$). The data indicate that productivity was affected indirectly through the influence of the latter on height, fat content and $V_{\max}$ (Spurr *et al.*, 1977a). Energy expenditure in men cutting sugar cane was measured along with food consumption. Energy intake was on the average 2970 kcal/day, i.e., 12.42 MJ/day. The estimated average energy expenditure over an 8-hr working day was 2254 kcal, i.e., 9.43 MJ. The men worked in the field, during the day with about 35% of maximum oxygen uptake and with about 57% during test periods.

Taller and heavier men were better workers and had lower heart rates at the mean working rate (Spurr *et al.*, 1975). The comparison of normally nourished and undernourished adult male subjects during a maximal oxygen uptake test showed that increased severity of malnutrition was associated with an increased

heart rate response to the same submaximal work loads. The response was observed to decrease during a period of high-protein dietary repletion of the most severely undernourished subjects (Spurr *et al.*, 1979).

Work performance was also better in Indian subjects with higher values of height and total and lean body mass (Satyanarayana *et al.*, 1978). Another measurement showed that the productivity (metric tons/day) of the Colombian sugar cane loaders was higher in men with less body fat, indicating a positive relationship between working productivity and physical fitness (Spurr *et al.*, 1977b). Poor nutrition also caused a lower working capacity and lower working potential, mainly because of a reduced lean body mass. The oxygen debt appeared sooner, and there was also a lower tolerance to oxygen debt (Viteri, 1976). Energy intake, lean body mass, hemoglobin, and aerobic capacity expressed as oxygen uptake during a maximal work load were closely interrelated, indicating the importance of the optimal level of morphological, nutritional, and functional factors for working performance (Viteri *et al.*, 1971).

Energy and protein supplementation improved working capacity: Guatemalan agricultural workers were given 5.5 g supplementary protein twice daily, 6 days a week for 3 years together with a higher total energy intake, compared to a group with lower energy intake and without protein supplementation. Lean body mass and muscular as well as adipose tissue were superior in the better nourished group, as was aerobic capacity. The supplemented group worked harder and completed tasks more quickly (Viteri and Torún, 1975). Nutritional repletion significantly improves body composition (increase in muscle mass, plasma volume, adipose tissue) of severely malnourished men (Barac-Nieto *et al.*, 1979), which is important for better work performance (DeMayer, 1975).

In men engaged in heavy work, the low intakes of both energy and proteins resulted in a negative nitrogen balance. Urinary excretion of hydroxyproline was influenced by the amount of dietary protein but not of energy (Iyengar and Rao, 1979).

The dietary intakes in various population groups may differ markedly, as in two villages in New Guinea (coastal and highland village, Norgan *et al.*, 1974). Work capacity and body morphology were also studied in Western Zaire (Austin *et al.*, 1979). Height and weight differed markedly in male and female Bantu, Ntomba, and Pygmoid Twa, but skin fold thickness and limb proportions were similar. Maximal oxygen uptake measured on a bicycle ergometer (derived indirectly from heart rates) showed differences in the absolute values, but maximal oxygen uptake per kilogram of body weight did not differ. Apparently, both aerobic capacity and relative body composition can be very similar in different ethnic groups, indicating also a similar level of physical fitness.

It is assumed that physical fitness is related to physical activity. But in ethnic groups characterized by a high level of physical fitness such as Eskimos or

New Guineans, the spontaneous motor activity is not markedly higher than in many subjects from industrially developed countries. Similar levels of fitness have been found in groups with very different levels of energy expenditure. These differences are most often explained by genetic and hereditary factors, natural selection, etc. The measurements of two New Guinean populations (again coastal and highland) showed differences in work performance that were not associated with the differences in level of physical activity. This was also true for shipyard workers in medium and heavy occupations compared to New Guineans, where the differences in fitness were more striking (Norgan and Ferro-Luzzi, 1978).

Undernutrition brings about adaptations including a reduction of physical activity. This was documented in field studies as summarized above (Keys *et al.*, 1950) and in those by Keller and Kraut (1962). It is difficult, therefore, to differentiate the effect of undernutrition and physical activity. In reverse situations, as in the case of Bantu mine workers, the initial months of employment were accompanied by both an increased food intake (4000 kcal/day, i.e., 16.73 MJ/day) and regular moderate physical work (Wyndham *et al.*, 1966), and their maximal oxygen uptake increased. In a more extreme case, i.e., obesity (as a special type of malnutrition), a limitation of work capacity also occurs which applies to all age categories. Norgan and Ferro-Luzzi (1978) reported that in obese adult Italian shipyard workers the maximal oxygen uptake expressed in milliliters per kilogram lean body mass per minute was the same as in normal subjects. Nevertheless, this was not true in adolescents in whom maximal oxygen uptake (milligram per kilogram lean body mass per minute) was significantly lower in the obese (Pařízková, 1977).

3.2.3. The Impact of Anemia

Deficiencies of special components of the diet, e.g., iron, resulting in anemia, have a decisive impact on work performance as mentioned in connection with experimental animals (Edgerton *et al.*, 1977). Reduced physical working capacity (Gardner, *et al.*, 1977) and lower productivity (Basta and Churchill, 1974; Basta, 1978) due to anemia were found. Significant decrease in performance was noted in those human subjects whose hemoglobin levels were below 12.0 g/100 ml blood compared to those with levels above 12.0 g/100 ml, and also in those individuals below 7.00 g/100 ml blood compared to those between 8.0 and 12.0 g/100 ml blood (Gardner *et al.*, 1975). Working capacity in anemic agricultural workers improved significantly in 2 months after supplementation of iron (Viteri and Cifuentes, 1973). Iron deficiency anemia in workers in tea plantations in Sri Lanka caused lower productivity which also improved after 1 month of iron supplementation treatment. Significantly more tea was picked when the hemoglobin concentration was increased. The degree of improvement

was greater in more anemic subjects, i.e., those with concentrations of 6.0 to 9.0 g/100 ml blood. Also, the spontaneous motor activity increased markedly after iron supplementation compared to placebo-matched subjects (Edgerton *et al.*, 1979), as did with maximal oxygen uptake (Chira *et al.*, 1979).

Marked and immediate improvement in performance capacity after an infusion of whole blood to severely anaemic subjects was observed (Edgerton *et al.*, 1975). Reduction of the heart rate at a given work load in response to iron therapy in iron-deficient subjects occurs even before a significant rise in hemoglobin. A comparison of subjects treated with iron dextran, whole blood, and untreated, all with a similar hemoglobin level, showed that the response to a work load was significantly lower after treatment with iron dextran than in subjects transfused with the whole blood or in untreated subjects. This suggests that iron treatment reduces the stress of physical activity more than can be expected from the improvement in the oxygen-carrying capacity of blood alone (Chira *et al.*, 1978).

3.2.4. Interaction of Supplementation and Exercise

Torún *et al.* (1977) showed that daily intake of 0.5 g/kg egg and mile protein was insufficient for men performing isometric exercise without training and caused negative nitrogen balance and a loss of body potassium, whereas 1 g/kg daily seemed to be adequate. Two other nonexercising men on a diet with 0.5 g/kg who lost weight had significant negative nitrogen balances and loss of body potassium which improved after their energy intake increased. The changes in nitrogen balance and body weight support the concept that protein requirements vary with energy intakes and, vice versa, that energy requirements are influenced by the level of protein intake.

3.2.5. Work Performance during Growth

The studies mentioned were mostly performed on adults. Studies of the functional capacity and altered performance in children caused by malnutrition are more rare (Cravioto, 1981). Kraut (1971) mentioned that the nutritional status of children in a developed country can be judged from their spontaneous motor activity. Passive immobility always signals a nutritional deterioration, just as high levels of spontaneous physical activity are a natural characteristic during growth, especially before puberty. Graves (1976) showed that undernourished children 7 to 18 months old show less vigor in play activity and less attachment behavior toward mother, especially in interaction across a distance.

Most experimental studies in children concern morphological and psychological development and were reviewed elsewhere. It is well known that body size, height, weight, weight for height or age, and skeletal and muscular devel-

opment (Widdowson, 1969) during growth are retarded in malnourished subjects. Skeletal development (Acheson *et al.*, 1977) is reduced because of intra-uterine malnutrition (Roord *et al.*, 1978). Bone age is retarded as well (Puri and Khanna, 1976) in preschool age and later in development (Waterlow and Alleyne, 1971).

In children with kwashiorkor and marasmus, pneumoencephalograms showed different degrees of cortical and subcortical atrophy; their performance in Sell's test was poorer than those of normally nourished children (Marcondes *et al.*, 1973). In children with protein-energy malnutrition, muscle wasting, hypotonia, and hyporeflexia were found. Peripheral nerve conduction velocity was delayed, and the needle electromyogram was abnormal for most children (Sachdev *et al.*, 1971). Biopsy samples from Egyptian children showed that the muscle glycogen of children with kwashiorkor and marasmus approached normal values (Shukry *et al.*, 1972). Parra *et al.* (1973) found low values of muscle glycogen, creatine phosphatase, and adenosine triphosphate. The latter two values showed a positive linear correlation with energy intake per unit of body weight during recovery and realimentation. The ⁴⁰potassium content (eq/kg body weight) was significantly reduced in children with protein-energy malnutrition (Talwalkar *et al.*, 1971). Working capacity was decreased in malnourished Indian adolescents (National Institute of Nutrition, 1978).

Severe clinical forms of malnutrition are closely related to low motor activity and a reduced level of functional capacity along with growth retardation. On the other hand, moderate levels of physical activity may have beneficial effects on children recovering from protein-energy malnutrition, enhancing longitudinal and lean body mass growth (Torún *et al.*, 1979).

Children with milder forms of undernutrition, characterized by being outside a certain range of growth standards, are also considered as malnourished. The percentage of children considered small for age varies in different countries. When standards from developed countries are used for comparison, the percentage of retarded children is obviously higher. The severity of impairment by malnutrition depends not only on diet but on special features of the "nutritional individuality" (Widdowson, 1962): in a malnourished community, subjects of all ages, including children, are never all affected in the same manner. General experience shows that tiny, thin children are often quite healthy and fit. The mechanisms of these differences are not yet clear (Durnin, 1979).

3.2.6. Marginal Malnutrition

Ferro-Luzzi *et al.*, (1979) measured children of preschool age in different parts of Italy with a different economic level and food intake. Smaller children from poorer regions had better functional efficiency of their heart response to exercise (step test modified for children, see Pařízková, 1977). This corre-

sponded to previous measurements of 11- to 12-year-old children in Tunisia (Pařízková and Merhautová, 1970). The results of the step test, vital capacity, performance in selected disciplines (running, broad jump, ball throwing, etc.), and absolute and relative muscle strength were also in most instances significantly better or at least the same in smaller children from poorer families than in those from well-off families with abundant diet and body size corresponding more to European standards. Comparison of Czech children, both light, asthenic and/or heavy or even obese led to the same conclusions: physical performance and especially the economy of work of the heart during a work load was better in those adolescents with lower body weight (Pařízková, 1977).

Relative aerobic capacity, that is, maximal oxygen uptake per kilogram of body weight, was significantly higher in Moscovian adolescents after World War II than in 1968 (Guminskyi *et al.*, 1972), when food intake, especially that of proteins, body weight, height, etc. were higher. Similar conclusions can be drawn from the comparison of adults measured before World War II and recently. Relative aerobic capacity as an indicator of cardiorespiratory fitness does not seem to increase along with absolute body size. Norgan and Ferro-Luzzi (1978) found quite high levels of physical fitness, tested during submaximal work loads, in adult New Guinean subjects with reduced energy intake levels [35 kcal/kg for men (0.14 MJ), 31 kcal/kg for women (0.129 MJ)]. Performance tests carried out by the Ministry of Education in Japan some time ago and recently showed that children with higher weight and height do not always display better results than those achieved by smaller children, particularly in endurance running (Suzuki *et al.*, 1978), which is an important indicator of cardiorespiratory fitness. Also in adults, good fitness was found in spite of quite reduced diet (Day *et al.*, 1976).

Podlešák (1979) tested miners and dock workers in India and foundry workers in Egypt in optimum climatic conditions using the methods recommended for the investigation of physical fitness by the Human Adaptability Section of the International Biological Programme. Generally, a lower work performance as defined by energy expenditure (W for 1 min, mean value from the whole shift) was found in Indian miners (see Figure 2) than in foundry workers of Egypt or standard values of Czechoslovak workers. Body weight and height were lower in Indian miners as compared to Europeans by 30 to 25%. Vital capacity was also 70 to 75% of European standards, and the recovery after work load was slower (ILO, 1967). Nevertheless, the level of aerobic capacity characterizing cardiorespiratory fitness, expressed as maximum oxygen uptake per kilogram body weight, was very good (Figure 3) in Indian miners in spite of poor nutrition and unsatisfying social-economic conditions resulting in reduced somatic development. The physical fitness of the subjects investigated was not, of course, representative of the population as a whole. All mentioned workers were trained for physical load, and after the medical examination, only healthy men were tested.

ENERGY EXPENDITURE FOR 1MIN.
MEAN VALUE FROM THE WHOLE SHIFT

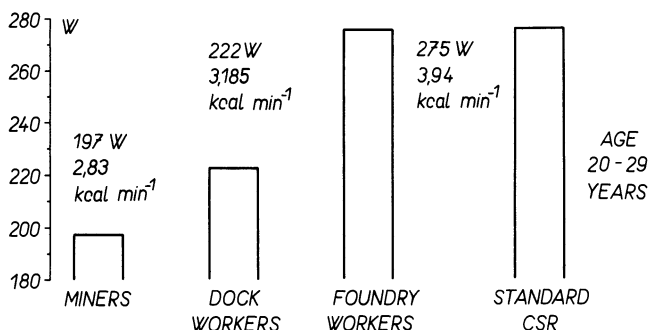


Figure 2. Work performance as defined by energy expenditure and watts per 1 min in Indian miners, dock workers, Egyptian foundry workers, and standard workers from Czechoslovakia.

In the general medical examination, interest was focused on blood picture, hemoglobin in the blood, and on parasites; subjects with schistosomiasis (Egypt) and with acute forms of *ankilostoma duodenale* or *entamoeba histolitica* were excluded. The basic anthropometric data were in good relation to the findings of other authors, especially in the case of Indian workers (see Podlešák, 1979).

These measurements show, however, that healthy subjects with reduced food intake and body dimensions adapted to physical work can have a good level of the aerobic capacity of the organism. In this case, it is again necessary to differentiate which aspect of fitness and performance is considered: the conclu-

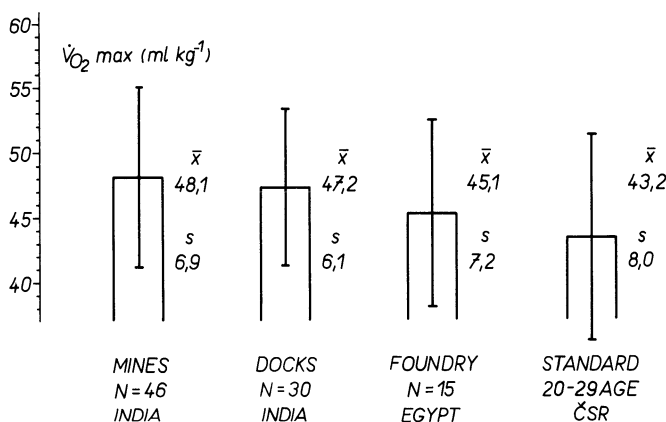


Figure 3. Aerobic capacity (expressed as maximal oxygen uptake per minute) related to body weight in Indian miners and dockers, Egyptian foundry workers, and Czechoslovak standard workers.

sions are not the same for absolute amount of work performed, especially that requiring greater absolute strength, as for cardiorespiratory efficiency and dynamic work of aerobic character.

4. CONCLUSIONS

Malnutrition interferes with a good level of work performance both when the subject suffers from energy deficiency or excess, not corresponding to his energy needs. In conjunction with that, it is necessary to differentiate various forms of malnutrition and undernutrition as well as energy deficiency and also various aspects of work performance.

Severe forms of clinical malnutrition cause marked deterioration of work performance, especially when the malnutrition is long lasting and starts early in the ontogeny or even during intrauterine life. This results in reduction of body size and development of bodily tissues along with deterioration of functional capacity. Short-term energy deficiency or even complete deprivation of food has been manifested by a decrease in endurance and muscle strength, but the performance during submaximal work load was not influenced. Semistarvation in originally well-fed subjects that lasts at least several months mostly affected performance such as lifting, pushing, carrying loads, walking long distances, the ability to climb, but performance in speed and accuracy seemed to be less influenced. Aerobic capacity was also reduced.

Life-long restriction of energy and protein intake that is still within the adaptation limits of the organism does not necessarily lead to such results, and the undernourished subjects can sometimes be capable of very good physical performance. This is apparent from some field studies in developing countries and was shown very strikingly in underweight long-distance runners or gymnasts (especially females) who all are outside the range of usual weight and height standards. These subjects often have drastically reduced food intake and are still capable of very high physical performance. Genetic factors enabling the subject to cope with undernutrition must be considered as well; experience shows that marginal malnutrition to which the organism is adapted during long periods of life can manifest positively in certain aspects (Fanconi, 1969), e.g., in cardiorespiratory efficiency.

Animal experiments show that animals fed reduced diets during prolonged periods of life survive longer, develop higher levels of spontaneous motor activity and good performance capacity, and are more resistant to certain noxi causing necrosis of the heart muscle. Studies in long-lived individuals showed that they were poorly nourished with regard to energy and proteins in childhood (Hejda, 1969). The ineffectual increase in the economic level of industrially developed countries with a too abundant and disbalanced food intake not only

does not always lead to an increased level of working capacity and physical fitness as well as good health ("civilization diseases") but is also somewhat costly: expenses for food are at least ten times higher than in developing countries (Behar, 1976a,b). An optimum food intake, or "optimal minimum" related to the criteria of desirable fitness, both mental and physical, health, and survival ought to be studied experimentally and defined in relation to both local and world economic resources.

REFERENCES

- Acheson, R., Canosa, C. A., and Blanco, R., 1977, Nutrition and bone development, *Rev. Esp. Paediatr.* 33:583–590.
- Adams, P., 1969, The effect of experimental malnutrition on the development of long bones, *Bibl. Nutr. Dieta* 13:60–73.
- Anderson, G. D., Ahokas, R. A., Lipshitz, J., and Dilts, V., Jr., 1980, Effect of maternal dietary restriction during pregnancy on maternal weight gain and foetal birth in the rat, 110:883–890.
- Anderson, K. L., 1973, The effect of altitude variation on the physical performance capacity of Ethiopian men, I, II, in: *Physical Fitness*. (V. Seliger, ed.), Universitas Carolina, Prague, pp. 13–54.
- Asahina, K., 1977, Growth and work capacity of Japanese people, in: *Human Adaptability* (K. J. Collins and J. S. Weiner, eds.), Taylor and Francis, London, pp. 168–170.
- Åstrand, P. O., and Rodahl, K., 1977, *Textbook of Work Physiology*, McGraw Hill, New York.
- Austin, D. M., Ghesquiere, J., and Azama, M., 1979, Work capacity and body morphology of Bantu and Pygmoid groups of Western Zaire, *Human Biol.* 51:79–89.
- Baker, P. T., 1977, Andean biocultural studies, in: *Human Adaptability*, (K. J. Collins and J. S. Weiner, eds.), Taylor and Francis, London, pp. 291–293.
- Barac-Nieto, M., Spurr, G. B., Lotero, H., Maksud, M. G., and Dahners, H. W., 1979, Body composition during nutritional repletion of severely undernourished men, *Am. J. Clin. Nutr.* 32:981–991.
- Barbashova, Z. I., 1977, Adaptation to the circumpolar region in USSR, in: *Human Adaptability* (K. J. Collins and J. S. Weiner, eds.), Taylor and Francis, London, pp. 322–325.
- Basta, S. S., 1978, Effect of anemia, *Am. J. Clin. Nutr.* 31:197–198.
- Basta, S. S., and Churchill, A., 1974, Iron deficiency anemia and the productivity of adult males in Indonesia, *World Bank Staff Paper No. 175*, World Bank, Washington D.C.
- Behar, M., 1976a, La nutricion, Un problema social, *Bol. Of. Sanit. Panam.* 80:533–539.
- Behar, M., 1976b, Nutrition and the future of mankind, *J. Health Serv.* 6:315–320.
- Bhavani, B., 1966, Nutrition and efficiency in agricultural labourers, *Indian J. Med. Res.* 54:971–976.
- Bosco, J. S., Greenleaf, J. E., Bernauer, E. M., and Card, D. H., 1974, Effect of acute dehydration and starvation on muscular strength and endurance, *Acta Physiol. Pol.* 25:411–424.
- Boyo, A. E., 1977, Nutritional, metabolic, and genetic studies in West African groups including children, in: *Human Adaptability*, (K. J. Collins and J. S. Weiner, eds.), Taylor and Francis, London, pp. 225–226.
- Cahill, C. F., 1970, Starvation in man, *N. Eng. J. Med.* 282:668–675.
- Chakraborty, M. K., and Guha Roy, A. R., 1966, Aerobic work capacity in Indian miners, in: *Human Adaptability* (M. S. Malhotra, ed.), Defence Institute of Physiology and Allied Sciences, Madras, pp. 107–119.

- Cravioto, J., 1981, The W. O. Atwater Memorial Lecture: "Nutrition, stimulation, mental development, and learning", in: *Proceedings of the Twelfth International Congress on Nutrition*, San Diego, in press.
- Daws, T. A., Consolazio, F. C., Hilty, S. L., Johnson, H. L., Krzywicki, H. J., Nelson, R. A., and Witt, N. F., 1972, Evaluation of pulmonary function and work performance in man during caloric restriction, *J. Appl. Physiol.* 33:211–217.
- Day, J., Carruthers, M., Bailey, A., and Robinson, D., 1976, Anthropometric, physiological and biochemical differences between urban and rural Masai, *Atherosclerosis* 23:357–361.
- DeMayer, E. M., 1975, Target groups for protein-rich mixtures, in: *Nutrition, Proceeding 9th International Congress on Nutrition, Mexico 1972*, Volume 4, Karger, Basel, pp. 159–164.
- Durnin, J. V. A. G., 1979, Energy balance in man with particular reference to low intakes, *Bibl. Nutr. Dieta* 27:1–10.
- Edgerton, V. R., Hettiarachchi, J., Senawitne, B., Barnard, R. J., Gardner, G. W., Ohira, Y., 1975, Immediate work tolerance improvement with infusion of whole blood, in: *Xth International Congress on Nutrition, Kyoto, Japan, Aug. 3–9, 1975*, (Publication Committee of the Xth International Congress of Nutrition, eds.), The Science Council of Japan, Kyoto, Japan, p. 174.
- Edgerton, V. R., Diamond, L. B., and Olson, V., 1977, Voluntary activity, cardiovascular and muscular response to anemia to rats, *J. Nutr.* 107:1595–1601.
- Edgerton, V. R., Gardner, G. W., Ohira, Y., Gunawardena, K. A., and Senawiratne, B., 1979, Iron deficiency anemia and its effect on worker productivity patterns, *Br. J. Med.* 2:1546–1549.
- Elsner, R., 1966, Comparative physical fitness of American Indians and Caucasians, in: *Human Adaptability* (M. S. Malhotra, ed.), Defense Institute of Physiology and Allied Sciences, Madras, pp.156–160.
- Fanconi, G., 1969, Has malnutrition only bad consequences? What is the definition of health? in: *Protein–Calorie Malnutrition* (A. von Muralt ed.), Nestle Foundation, Springer Verlag, Berlin, Heidelberg, New York, pp. 57–62.
- FAO, 1962, *Nutrition and Working Efficiency, FFHC Basic Study No. 5*, FAO, Rome.
- Ferro-Luzzi, A., D'Amicis, A. D., Ferrini, A. M., and Maiale, G., 1979, Nutrition, environment and physical performance of preschool children in Italy, *Bibl. Nutr. Dieta* 27:85–106.
- Fleagle, J. G., Saunders, K. W., and Hegsted, D. M., 1975, Physical growth of cebus monkeys, *Cebus albifrons* during protein or calorie deficiency, *J. Clin. Nutr.* 28:246–253.
- Gardner, G. W., Edgerton, V. R., Barnard, R. J., and Senawiratne, B., 1975, Physical working capacity and iron deficiency in man, in: *Xth International Congress on Nutrition, Kyoto, Japan, Aug. 3–9 1975*, (Publication Committee of the Xth International Congress of Nutrition, eds.), The Science Council of Japan, Kyoto, Japan, p. 174.
- Gardner, G. W., Edgerton, V. R., Senawiratne, B., Barnard, R. J., and Ohira, Y., 1977, Physical work capacity and metabolic stress in subjects with iron deficiency anemia, *Am. J. Clin. Nutr.* 30:910–917.
- Gelfand, M., Jones, J. J., and Thomas G. E., 1975, Nutritional microcardia. Recovery of heart size and function with treatment, *Cent. Afr. J. Med.* 21:167–171.
- Ghesquierre, J., and Anderson, K. L., 1973, Similarities and differences in physiological responses to muscular exercise among primitive African men of two genetically different population groups, in: *Physical Fitness* (V. Seliger, ed.), Universitas Carolina, Prague, pp. 164–176.
- Graves, P. L., 1976, Nutrition, infant behavior, and maternal characteristics: A pilot study in West Bengal, India, *Am. J. Clin. Nutr.* 29:305–319.
- Guminskyi, A. A., Elizarova, O. S., Zhurkov, N. N., Zolotayko, G. A., and Novozhilova, A. D., 1972, [On functional acceleration of the actual generation], *Pediatrriia* 3:6–8.
- Hegerty, P. V., and Kim, K. O., 1980, Changes in skeletal muscle cellularity in starved and refed young rats, *Br. J. Nutr.* 44:123–127.

- Hejda, S., 1968, Ernährungsverhältnisse während der Kindheit bei langlebenden Personen. *Z. Alternsforsch.* 21:159–165.
- Himes, J. H., 1978, Bone growth and development in protein–calorie malnutrition, *World Rev. Nutr. Diet.* 28:143–187.
- Hornabrook, K. V., 1977, Human biology in New Guinea, in: *Human Adaptability* (K. J. Collins and J. S. Weiner, Eds.), Taylor and Francis, London, pp. 40–41.
- ILO, 1967, Report to the Government of India on Physiology in Mines, International Labor Office, United Nations Development Program, Technical Assistance Sector, TAP (India) R. 23, Geneva.
- Iyengar, A., and Rao, B. S. H., 1979, Effect of varying energy and protein intake on nitrogen balance in adults engaged in heavy manual labour, *Br. J. Nutr.* 41:19–25.
- Keller, W., and Kraut, H., 1962, Work and nutrition, in: *World Review of Nutrition and Dietetics* (G. H. Bourne, ed.), Hafner Publishing, New York, pp. 65.
- Keys, A., and Brožek, J., 1953, Body fat in adult man, *Physiol Rev.* 33:245–232.
- Keys, A., Henschel, A., and Taylor, H. L., 1947, The size and function of the human heart at rest in semistarvation and in subsequent rehabilitation, *Am. J. Physiol.* 150:153–169.
- Keys, A., Brožek, J., Henschel, A., Mickelsen, O., and Taylor, H. L., 1950, *The Biology of Human Starvation*, University of Minnesota Press, Minneapolis, pp. 601–606, 607–643, 689–713, 714–748.
- Kozioł, B. J., Ohira, R., Simpson, D. R., and Edgerton, V. E., 1978, Biochemical skeletal muscle and hematological profiles of moderate and severely iron deficient and anemic adult rats, *J. Nutr.* 108:1306–1314.
- Kraut, H., 1971, Report of synthesis and discussion, in: *Alimentation et Travail, Ier Symposium International, Vitell, Mai 1971* (G. Débry and R. Blayer, eds.), Masson et Cie., Paris, pp. 105–109.
- Kyger, E. R. III., Block, W. J., Roach, G., and Dudrick, S. J., 1978, Adverse effects of protein malnutrition on myocardial function, *Surgery* 84:147–156.
- Lahiri, S., 1977, Cardiorespiratory function and physical performance capacity of native residents of Nepal at various altitudes, in: *Human Adaptability* (K. J. Collins and J. S. Weiner, eds.), Taylor and Francis, London, pp. 136–138.
- Lee, M., and Mayers, G. S., 1979, The effect of protein energy malnutrition on appositional bone growth in the rat, *Experientia* 35:824–825.
- Loewenstein, F. W., 1968, Nutrition and Working Capacity Efficiency with Special Reference to Tropics, Joint FAO/WHO/OAU South Regional Food and Nutrition Commission for Africa, special paper No. 3, Accra, Ghana.
- Low, R. B., and Cerauskis, P. W., 1977, Biosynthesis of muscle protein in the fasted rats, *J. Nutr.* 107:1244–1254.
- Maitra, S. R., Chatterjee, S., and Das, S., 1966, Study in protein concentration in plasma and corpuscular changes of blood in men with submaximal and maximal work, in: *Human Adaptability*, (M. S. Malhotra, ed.), Defense Institute of Physiology and Allied Sciences, Madras, p. 130–140.
- Marcondes, E., Lefevre, A. B., Machado, D. V. M., Barros, N. G. De., Cavallo, A., Goral, S., Quarenti, G., Setian, N., Valente, M. J., and Barbiczi, D., 1973, Neuropsychomotor level and pneumoencephalogram changes in children with severe malnutrition, *J. Trop. Med. Environ. Child Health* 19:135–139.
- McCance, R. A., 1963, The bearing of early malnutrition on later development, in: *Proceedings of the VIth International Congress on Nutrition*, Edinburgh, pp. 74–81.
- Moore, F. D., Olesen, K. H., McMurray, J. D., Parker, H. V., Ball, M. R., and Boyden, C. M., 1963, The body cell mass and its supporting environment, in: *Body composition in health and disease*, W. B. Saunders, Philadelphia, pp. 3–11; 18–41.

- Nakono, K., 1978, Function of dietary protein, carbohydrate and fat *in vitro* protein synthesis in skeletal muscle of rats, *Nutr. Rep. Int.* 18:453–464.
- National Institute of Nutrition, 1978, *Report for 1977 of NIN*, Indian Council of Medical Research, Hyderabad, pp. 139–142.
- Nitzam, M., and Wilber, J. F., 1974, Effect of postnatal malnutrition on plasma proteins and growth, *Horm. Res.* 5:167–172.
- Nnanyelungo, D. O., 1978, Marginal congenital malnutrition on skeletal muscle composition, *Growth*, 42:167–177.
- Nolen, G. A., 1972, Effect of various restricted dietary regimens on the growth, health and longevity of albino rats, *J. Nutr.* 102:1477–1993.
- Norgan, N. G., and Ferro-Luzzi, A., 1978, Nutrition, physical activity and physical fitness in contrasting environments, in: *Nutrition, Physical Fitness and Health* (J. Pařízková, and V. A. Rogozkin, eds.), University Park Press, Baltimore, pp. 167–193.
- Norgan, N. G., Ferro-Luzzi, A., and Durnin, J. V. A. G., 1974, The energy and nutrient intake and the energy expenditure of 204 New Guinean adults, *Phil. Trans. R. Soc. Lond. Biol.* 268:309–348.
- Ohira, Y., Edgerton, V. E., Gardner, G. W., Senawiratne, B., and Simpson, D. R., 1978, Non-hemoglobin related effects of heart rates in iron deficiency anemia, *Nutr. Rep. Int.* 18:647–651.
- Ohira, Y., Edgerton, V. E., Gardner, G. W., Senawiratne, B., and Banard, R. J., 1979, Work capacity, heart rate and blood lactate responses to iron treatment, *Br. J. Hematol.* 41:365–372.
- Pařízková, J., 1977, *Body Fat and Physical Fitness*, Martinus Nijhoff, The Hague.
- Pařízková, J., 1978a, Nutrition and functional capacity, *Proc. Nutr. Soc. India* 22:25–38.
- Pařízková, J., 1978b, Malnutrition and physical performance, in: *Practical Approaches to Combat Malnutrition with Special Reference to Mothers and Children*, Cairo, Egypt, IUNS Conference Proceedings (N. S. Scrimshaw and M. Gabr, eds.), Ministry of Health, Cairo, pp. 101–131.
- Pařízková, J., 1978c, The impact of nutrition and work load during pre- and postnatal life on later development, *WHO Chron.* 32:204.
- Pařízková, J., 1979, *The Assessment of Nutrition, Particularly in Regard to Protein Energy Malnutrition in Adults, and with Special Reference to Pregnant and Lactating Women*, World Health Organization, Nutrition Unit, Geneva.
- Pařízková, J., 1981, Physical activity related to nutrition as a factor of variability of body composition, in: *Food, nutrition and evolution*. (D. N. Walcher and N. Kretchmer, eds.), Masson, New York, pp. 133–141.
- Pařízková, J., and Merhautova, J., 1970, The comparison of somatic, development, body composition and functional characteristics in Tunisian and Czech boys 11 and 12 years old, *Hum. Biol.* 42:391–400.
- Pařízková, J., and Petrásek, R., 1979, Impact of early malnutrition on later development of spontaneous physical activity and lipid metabolism, *Nutr. Metab.* 23:266–274.
- Pařízková, J., Petrásek, R., and Fraňková, S., 1979, The impact of reduced energy and protein intake at the beginning of life on growth, spontaneous motor activity and lipid metabolism in male rats, *Indian J. Nutr. Diet.* 16:412–416.
- Pařízková, J., Faltová, E., Mráz, M., and Špátová, M., 1982, Growth, food intake, motor activity and experimental cardiac necrosis in malnourished rats, *Ann. Nutr. Metab.* 26:121–128.
- Parra, A., Garza, C., Garza, Y., Saravia, J. L., Harlewood, C. F., and Nichols, B. L., 1973, Changes in growth hormone, insulin and thyroxin values and in energy metabolism in marasmic children, *J. Pediatr.* 82:133–142.
- Podlešák, K., 1979, Physical fitness of Indian and Egyptian workers and permissible level of work output, *Arh. Hig. Rada Toksikol.* 30 (Suppl. 4):1719–1723.
- Pratt, C. C. W. M., and McCance, R. A., 1964, Severe undernutrition in growing and adult animals. 12. The structure of the long bones in pigs, *Br. J. Nutr.* 18:393–408.

- Puri, R. K., and Khanna, K. K., 1976, Skeletal changes in protein energy malnutrition, *Indian Pediatr.* 13:833–836.
- Raju, J. J., 1974, Effect of early malnutrition on muscle function and metabolism in rats, *Life Sci.* 15:949–960.
- Roord, J. J., Ramaekers, L. H. J., and Engelshoven, J. M. A. Van, 1978, Intra-uterine malnutrition and skeletal retardation, *Biol. Neonate.* 34:167–169.
- Roy, J. K., 1966, Diet surveys on some aboriginal tribes in India, in: *Human Adaptability* (M. S. Malhotra, ed.), Defense Institute of Physiology and Allied Sciences, Madras, pp. 195–209.
- Sachdev, K. K., Taori, G. M., and Pereira, S. M., 1971, Neuromuscular status in protein energy malnutrition. Clinical, nerve conduction and electromyographic study, *Neurology (Minneapolis)* 21:801–805.
- Salas, M., and Cintra, L., 1975, Development of the electrocorticogram during starvation in the rat, *Physiol. Behav.* 14:589–593.
- Satyanarayana, K., Nadamuni Naidu, A., Chatterjee, B., and Narasinga Rao, B. S., 1978, Nutritional status, body physique and work output, in: *Nutrition, Physical Fitness and Health* (J. Pařízková and V. A. Rogozkin eds.), University Park Press, Baltimore, pp. 215–226.
- Shrader, R. E., and Zeman, F. J., 1973, Skeletal development in rats affected by maternal protein deprivation and postnatal food supply, *J. Nutr.* 103:792–801.
- Shukry, A. S., Fayad, I. M., Eisa, E. A., and Salem, S. J., 1972, Phosphorylase activity in the liver and muscle tissue in PCM. *J. Trop. Med. Hyg.* 75:240–245.
- Sirotnin, N. N., and Matsynin, V. V., 1977, Adaptation to high altitude, in: *Human Adaptability* (K. J. Collins and J. S. Weiner, eds.), Taylor and Francis, London, pp. 314–316.
- Spurr, G. B., Barac-Nieto, M., and Maksud, M. G., 1975a, Energy expenditure cutting sugarcane, *J. Appl. Physiol.* 39:990–996.
- Spurr, G. B., Barac-Nieto, M., and Maksud, M. G., 1977a, Productivity and maximal oxygen consumption in sugar cane cutters, *Am. J. Clin. Nutr.* 30:316–321.
- Spurr, G. B., Maksud, M. G., and Barac-Nieto, M., 1977b, Energy expenditure, productivity and physical work capacity of sugarcane loaders, *Am. J. Clin. Nutr.* 30:1740–1746.
- Spurr, G. B., Barac-Nieto, M., and Maksud, M. G., 1979, Functional assessment of nutritional status: Heart rate response to submaximal work, *Am. J. Clin. Nutr.* 32:767–778.
- Srivastava, U., Vu, M. L., and Goswami, T., 1974, Maternal dietary deficiency and cellular development of progeny in the rat, *J. Nutr.* 104:512–520.
- Stewart, R. J. C., 1976, Twelve generations of marginal malnutrition, *Baroda J. Nutr.* 3:145–155.
- Stickland, N. C., Widdowson, E. M., and Goldspink, G., 1975, Effect of severe energy and protein deficiencies on the fibres and nuclei in skeletal muscle of pigs, *Br. J. Nutr.* 34:421–428.
- Stini, W. A., 1981, Body composition and nutrient reserves in evolutionary perspective, in: *Food, nutrition and evolution*, (D. N. Walcher and N. Kretchmer, eds.), Masson, New York, pp. 107–120.
- Ström, G., 1962, Methods for the determination of physical capability, in: *Mild-Moderate Forms of Protein-Energy Malnutrition* (G. Blix, ed.), Swedish Foundation, pp. 127–130.
- Suzuki, S., Oshima S., Tsuji, E., Tsuji, K., and Ohta, F., 1978, Interrelationships between nutrition, physical activity and physical fitness, in: *Nutrition, Physical Fitness and Health* (J. Pařízková and V. A. Rogozkin, eds.), University Park Press, Baltimore, pp. 194–214.
- Talwalkar, Y. B., Gupta, S. C., Hukko, R. K., Doude, V. M., and Bharucha, P. E., 1971, Total body potassium in protein-energy malnutrition, *Indian Pediatr.* 8:686–690.
- Torún, B., Scrimshaw, N. S., and Young, V. R., 1977, Effect of isometric exercises on body potassium and dietary protein requirements of young men, *Am. J. Clin. Nutr.* 30:1983–1993.
- Torún, B., Schutz, Y., Viteri, F., and Bradfield, R. B., 1979, Growth, body composition and heart rate/ VO_2 relationship changes during the nutritional recovery of children with two different physical activity levels, *Bibl. Nutr. Dieta* 27:55–56.

- Viteri, F., 1976, Definition of the nutrition problem in the labor force, in: *Nutrition and Agricultural Development* (N. S. Scrimshaw and M. Behár, eds.), Plenum Press, New York, pp. 87–98.
- Viteri, F., and Cifuentes, E., 1973, Capacidad física, deficiencia de hierro y anemia en trabajadores agrícolas de Centro America, in: *XV. Congreso Médico Centroamericano, San José, Costa Rica, Dec. 4–8*.
- Viteri, F., and Torún, B., 1975, Energy intake and physical work capacity of agricultural labourers in Guatemala. Effect of dietary supplementation and its role in health programmes, *Bol. Of. Sanit. Panam.* 78:58–74.
- Viteri, F., Torún, B., Galicia, J. C., and Herrerra, E., 1971, Determination of energy costs of agricultural activities by respirometer and energy balance techniques, *Am. J. Clin. Nutr.* 13:1418–1430.
- Waterlaw, J. C., and Alleyne, G. A. O., 1971, Protein malnutrition in children: Advance in knowledge in the last ten years, *Adv. Protein. Chem* 25:117–241.
- Weiner, J. S., and Laurie, J. A. (eds.), 1969, *Human Biology, A Guide to Field Methods*, IBP Handbook No. 9, Blackwell Scientific, Oxford, Edinburgh.
- Widdowson, E. M., 1962, Nutritional individuality, *Proc. Nutr. Soc.* 21:121–128.
- Widdowson, E. M., 1964, Early malnutrition and later development, in: *Diet and Bodily Constitution*, CIBA Foundation, Study Group No. 17, Churchill, London, pp. 3–10.
- Widdowson, E. M., 1969, Changes in the extracellular compartment of muscle and skin during normal and retarded development, *Bibl. Nutr. Dieta* 13:60–68.
- Widdowson, E. M., and McCance, R. A., 1956, The effects of chronic undernutrition and of total starvation on growing and adult rats, *Br. J. Nutr.* 10:363–373.
- Winick, M., 1970, Nutrition and mental development, *Med. Clin. North Am.* 54:1413–1429.
- Wyndham, C. H., 1977, Measurement of the maximum oxygen intake of various South African Bantu groups stratified according to their degrees of urbanization, in: *Human Adaptability* (K. J. Collins and J. S. Weiner, eds.), Taylor and Francis, London, pp. 250–251.
- Wyndham, C. H., Strydom, N. B., Morrison, J. F., Bredell, C. G., and Heyns, H., 1966, The capacity for endurance effort of Bantu males from different tribes, *S. Afr. J. Sci.* 62:259.
- Yoshimura, H., and Weiner, J. S., (eds.), 1966, *Human Adaptability and Its Methodology. Proceedings, Symposium Held in Kyoto, Japan, 1965*, Japan Society for the Promotion of Sciences, Tokyo.

SUGGESTED READING

Energy requirements: How much is enough? 1980, *Nutr. Rev.* 38:337–338.

Multisectoral Nutrition Interventions

A Policy Analysis Perspective

BRUCE F. JOHNSTON

1. INTRODUCTION

The widespread and serious problems of malnutrition in developing countries are unquestionably the result of multiple causal factors. The fact that malnutrition, especially in developing countries, is to such a large extent a manifestation of poverty is sufficient to underscore the importance of multiple causation. In addition, I will be arguing that for the groups that are nutritionally most vulnerable—infants and small children and pregnant and lactating women—factors in addition to poverty are also of great importance.

Does the fundamental importance of multiple causal factors mean that we should rely on “multisectoral nutrition interventions” to achieve our goal of promoting better nutrition? The short answer is an emphatic “No.” Ruth Huenemann (1981, p. 124) has stated that the goal of nutrition advocates (exemplified by the American Dietetic Association) is “to have food and nutrients make their maximum contribution to human welfare.” Clearly, there are innumerable ways in which that goal can be advanced. Therefore, it is essential to adopt a broad, multisectoral perspective in identifying the policies and programs that will be feasible and most effective in furthering that nutritional goal. But it does not follow that interventions should be “multisectoral” in the sense of attempting to administratively integrate the activities of two or more sectoral agencies, e.g., health and agriculture. In fact, integrated or multisectoral pro-

grams in that sense should, in general, be avoided because they pose such difficult administrative problems.

The range and content of the interventions that will be feasible and effective obviously will depend on the characteristics, constraints, and opportunities found in a particular country. In a high-income industrialized country such as the United States, it is appropriate to pursue a wide range of special programs—a Food Stamp Program, the Supplemental Food Program for Women, Infants, and Children (WIC), the Expanded Food and Nutrition Education Program (EFNEP), food safety and nutrition labeling regulations, special programs for the elderly such as meals at senior centers, and many others. A low-income developing country obviously faces much more severe economic, budget, and manpower constraints, but the “need” for action to improve the nutritional status of its population is also much greater. Hence, the problems of determining priorities and making hard choices are both difficult and critically important. The program interventions that merit priority in such countries will differ drastically from those appropriate for a high-income, developed country.

It is all too apparent that the “universal declaration” for “the eradication of hunger and malnutrition” by 1985 which was adopted by the World Food Conference in 1974 will not be fulfilled. In fact, it is easy to dismiss that resolution as the meaningless rhetoric of an international conference. It is well to recall, however, the climate of opinion that prevailed in 1974. The “nutrition factor” had just been discovered by the international development community (see Berg *et al.*, 1973; Berg, 1973; Joy, 1973; United Nations, 1975). And in some circles, extravagant hopes were placed in the new “discipline” of nutrition planning. To be sure, there were a variety of views of nutrition planning. At one extreme there was the very comprehensive approach which Field (1977) has referred to as the “intellectual establishment view.” At the opposite extreme, there was (and is) the “bureaucratic view” which focuses narrowly on specific nutrition interventions such as supplementary feeding programs and food fortification. I have argued elsewhere (Johnston, 1979) that these attempts to define a new and powerful role for “nutrition planners” capable of finding shortcuts to the promised land of a world free of hunger have failed.

My purpose is not to bewail the predictable failure of nutrition planning to find those shortcuts or the equally predictable inability of the “international community” to fulfill the universal declaration of 1974 to eliminate hunger and malnutrition by 1985. It should have been obvious even in 1974 that the elimination of malnutrition and related manifestations of poverty would depend much more on the decisions and action of governments in well over a hundred developing countries than on decisions by the international community acting through the UN system, supported by bilateral aid programs. My objective in this chapter is rather to derive lessons from past experience that will provide more valuable policy guidance in the future.

Given the enormously complex and inherently difficult problems of fostering development and of reducing malnutrition, mistakes and failures are inevitable. A major thesis of this chapter concerns the importance of emphasizing a more systematic trial-and-error learning process in acquiring the understanding and organizational capabilities required to cope more effectively with these problems. I occasionally like to recall that my first published article had one distinguishing feature: it was magnificently wrong. After working in Japan for 3 years with responsibilities for food rationing, price control, and food import programs in General MacArthur's SCAP Headquarters, I wrote a piece on "Japan: The Race Between Food and Population" (Johnston, 1949). To the great good fortune of the Japanese people, my pessimistic assessment proved to be totally mistaken. The Japanese economy, like any economy, is still confronting difficult problems. But the ability to feed its population is not one of them.

In the course of a year at the International Institute for Applied Systems Analysis (IIASA) in 1978–1979, I reached the conclusion that the art and craft of "policy analysis" provide a particularly useful perspective for reflecting on the complex, ill-structured problems that must be confronted if we are to make satisfactory progress in reducing the malnutrition and other severe consequences of poverty that are such widespread problems in most of the less-developed countries. One thing is certain: little will be accomplished if we continue to approach these problems solely from the perspective of our particular scientific discipline. In the Cooper Memorial Lecture, from which I have already quoted, Huenemann (1981, pp. 124–125) emphasizes that "nutrition is not only the concern of the scientist who advances knowledge but also that of the interpreter who makes it live." She goes on to quote a fine statement by Conrad Elvehjem which emphasizes that the future of scientific progress depends as much on "the interpreter of science" as on the scientist who advances knowledge by deep understanding of a narrow field in which he or she is an authority. It is my contention that if we, as "nutrition advocates," want to be effective in contributing to progress in attaining nutritional goals, we must become "policy analysts" and acquire the skills and understanding that are needed in order to exert a constructive influence on policy-making processes.

2. POLICY ANALYSIS AND THE ACHIEVEMENT OF NUTRITIONAL GOALS

There have been remarkably few attempts to apply a policy analysis perspective to the problems of development and none, to my knowledge, that have treated food and nutrition policy from that perspective. Policy analysis, as Clark and I have used the term and as it is used at the International Institute for Applied

Systems Analysis (IIASA), is really synonymous with systems analysis.* Both terms have, however, been used in many different ways. One of the ablest practitioners of the art and craft of policy analysis, W. Granger Morgan, felt constrained to use the label "good policy analysis" in describing the field in a guest editorial in *Science*. He states that the object of good policy analysis "is to evaluate, order, and structure incomplete knowledge so as to allow decisions to be made with as complete an understanding as possible of the current state of knowledge, its limitations, and its implications" (Morgan, 1978).

The design of development and of food and nutrition strategies must take into account a host of variables and complex and changing interrelationships among those variables. Hence, complete knowledge and understanding are impossible. In addition, judgments and decisions will inevitably be influenced by opinions, preferences, values, and vested interests. Nevertheless, as Morgan argues, good policy analysis can order and structure that incomplete knowledge "in ways that are open and explicit"; and it can and should avoid drawing "hard conclusions unless they are warranted by unambiguous data or well-founded theoretical insight."

I argue in Section 3 that some of the implications that can be derived from our knowledge of the economic, structural, and demographic characteristics of developing countries point to certain "hard conclusions." This is most likely to be true if we focus on a relatively homogeneous subset of developing countries, and it is partly for that reason that I concentrate in this chapter on the food and nutrition problems of low-income, late-developing countries. The World Bank (1980) currently defines the low-income developing countries as those with 1978 per capita GNP of \$360 or less. This subset corresponds fairly well with the subset of "late-developing" countries, a term I have used to describe those less developed countries that still have a predominantly agrarian structure and where some 60 to 80% of the labor force still depend on agriculture for a livelihood (Johnston and Kilby, 1975). Some of the conclusions that I advance in Section 3 are, however, tentative because they are based on evidence that is incomplete and somewhat ambiguous.

In outlining a perspective from policy analysis for considering the ill-structured problems of development, Clark (Johnston and Clark, 1982, Chapter 1) emphasizes that in this context policy analysis must focus on people and their interactions with each other and with their environment. These interactions are mediated through various organizations and their policies and programs. To

*This section draws heavily on my forthcoming book, *Redesigning Rural Development: A Strategic Perspective* (Johnston and Clark, 1982). I am deeply indebted to Clark for an extended tutorial in policy analysis. The ideas presented in this section are essentially a condensation and paraphrase of Clark's chapter on "Policy Analysis and the Development Debate" in our book. An earlier joint working paper (Johnston and Clark, 1979) and a paper that I prepared for an MIT conference (Johnston, 1979) also attempt to treat the issues that arise in development and in designing a food and nutrition strategy from a policy analysis perspective.

fulfill their functions, organizations rely on a variety of social techniques of calculation and control: markets and exchange systems, political organizations, bureaucracies, cooperatives, family and community groups, and many others. Policies and policymaking processes in the broadest sense can be viewed as social action programs through which people seek to devise and implement solutions to their problems. In fact, an important task of policy analysis as applied to ill-structured problems such as development and nutritional improvement is to convert a “mess” of overlapping problems into problems defined in such a way that something constructive can be done about them. Policymaking processes in this broad perspective embrace tactics of planning, of revolution, of government interventions, and of institutional reform.

Both planners and academics are prone to emphasize a “thinking-through” approach to problems and to the formulation of plans that are essentially “blue-prints” which, it is hoped, will somehow be adopted and implemented. In fact, policymakers seldom start with a “clean slate.” Policies are not so much designed as redesigned as modifications of existing policies in a continuing sequence of policy design and redesign. Hirschman (1971, pp. 19–20) puts it well: the critical need is to “think in terms of sequences in the course of which a forward step in one direction will induce others. . . .” Colin Leys (1971, p. 137) has argued similarly that “the process of ‘choice’ rarely consists of an explicit ‘moment’ at which some appropriate person or committee reviews the alternatives, weighs their pros and cons and consciously selects one of them.” The realistic challenge is to assess

what changes—social and political, as well as economic—are within the politicians’ “means,” and what are not; and what patterns or sequences of change, among those that are practical will carry the process of economic development farthest and fastest at the least cost in the politicians’ resources (Leys, 1971, p. 133).

There are four propositions suggested by a policy analysis perspective that appear to be especially relevant to the design and redesign of development, including policies and programs directed at the goal of improving nutritional status and health. These pertain to (1) power and persuasion, (2) feasibility and desirability, (3) inefficiency and the lack of consensus, and (4) cogitation and interaction.

2.1. Power and Persuasion

This first proposition simply emphasizes that if we want to be effective we must learn “to speak truth to power”—and speak it clearly and persuasively enough so that the goals of reducing malnutrition and related deprivations of poverty will be advanced to the maximum degree that is feasible.* Given the

*The phrase is taken from Milton and is part of the title of Wildavsky’s recent book, *Speaking Truth to Power: The Art and Craft of Policy Analysis* (1979).

complexity and uncertainty that characterize the messy problems associated with development and nutrition interventions, the “truth” that we are able to speak is bound to be partial and incomplete. I turn to that problem shortly.

A problem pointed up by the relationship between power and persuasion is to avoid a kind of naivete which is a frequent source of ineffectiveness and failure to make the progress that is possible within the limits of the feasible. It is often remarked that failures to help the poor are “essentially” political failures. That is one of the truest and least useful observations that can be made about development programs. Politics, power, and competing and conflicting interests are inevitable features of human societies. To bewail the unequal distribution of power is about as conducive to resolving “essentially political” problems as bewailing the unequal distribution of rainfall is to resolving “essentially agricultural” problems. If we are to function as effective policy analysts, we must learn to understand the constraints that must shape feasible programs, and those constraints are related to the world of power as much as to constraints imposed by nature and by the scarcity of economic, budget, and manpower resources.

A common pitfall is the presumption that if only the “right” political ideology could be incanted, malnutrition and other deprivations could be easily eliminated. To be sure, there may well be situations in which power is held so completely by vested interests totally opposed to advancing the interests of the poor and malnourished that the policy analyst is faced with a painful choice between getting power and getting out. Nicaragua under Somoza appears to have been a particularly clear example of such a situation. But let us not forget that getting rid of Somoza did not solve Nicaragua’s problems of poverty, malnutrition, and excessively high mortality and morbidity. It was only a first though seemingly necessary step toward designing and implementing the various policies and programs that, it is to be hoped, will eventually overcome those problems.

2.2. Feasibility and Desirability

Another common pitfall of development practioners, advisors, and academic development specialists is to equate the feasible with the desirable. And the equation is applied in both directions with equally unfortunate results.

On the one hand, many people assume that what they want they can get. Perhaps especially with a problem such as severe malnutrition, the goal of eliminating malnutrition is so desirable that we are tempted to assume that any measures directed toward that goal must be feasible as well. But in a world of economic and political constraints, the time, money, talent, “will,” and effort are never enough for all of the important tasks at hand.

On the other hand, some pragmatic politicians and advisors tend to reimplement policies that have worked in the past, i.e., demonstrably feasible activities.

This is, of course, better than retaining programs and policies that are unfeasible. But again, since we live in a world that is constrained by a scarcity of resources, it is essential to recognize that eventually one choice must preclude another, and choosing one alternative almost always means not doing something else.

In the jargon of economics, the opportunity cost of doing some things that are both feasible and desirable may be too high because they divert attention and resources away from other actions which might have a much greater impact. I believe, for example, that in low-income developing countries, school lunch programs have been too feasible. They have thus provided a convenient outlet for the urge to improve immediate nutritional status but have thereby diverted attention away from the search for more far-reaching alternatives. It is unfortunately the case that emphasis on feasible and visible activities such as school lunch programs and urban food subsidies has often preempted resources of money, scarce administrative capacity, and attention away from more fundamental programs. As policy analysts concerned with achieving the maximum results that are feasible, we should give a higher priority to building a support coalition for programs that enable the great bulk of a country's population, rural as well as urban, to have access to at least a minimum package of nutrition, health, and family-planning services.

There is a more general conclusion to be derived from this analysis of feasibility and desirability. The textbook approach to planning emphasizes a procedure whereby goals or objectives are first established, and then the means for attaining those objectives are determined. This is also true of some treatments of policy analysis (see, for example Stokey and Zeckhauser, 1978). There is an attractive logic about that approach, but it is in fact misleading, especially in dealing with ill-structured problems and in situations where insufficient resources severely limit the objectives that are feasible. Therefore, good policy analysis emphasizes the need for mutual adjustment of ends and means. That is, the definition of objectives must be adapted to the resources that are available, and vice versa, to the extent that available resources can be enlarged or deployed more effectively. Moreover, as I argue shortly, this mutual adjustment of ends and means requires a continuing, iterative process of policy design, implementation, and redesign as initial judgments about feasibility and desirability are modified in the light of actual experience.

2.3. Ineffectiveness and the Lack of Consensus

This third proposition concerns the ineffectiveness of much of the development debate and the related failure to build the degree of consensus required for effective action. One aspect has been the tendency to flit from fad to fad rather than to achieve cumulative progress toward better understanding and more effective performance. A related aspect has been to emphasize controversy and a

succession of key factors which have often been treated almost as panaceas. The early emphasis in investment and growth as the answer was followed by a burst of enthusiasm for investment in human resources and manpower planning, then a focus on expanding employment as the all-important need, and advocacy of a "basic needs strategy" has been the latest "new direction." In the field of nutrition, the concentration in the 1960s on "the protein gap" as the world's principal food problem and emphasis on products such as fish protein concentrate (FPC) has now given way to a similarly narrow focus on energy as the overriding problem. Whereas cassava was widely condemned by nutritionists in the 1960s since it is such a miserable source of protein, many are now extolling its virtues because of its high calorie yield per acre.

In a field as complex as development, controversy is inevitable, even useful. But the sort of skepticism and criticism that are appropriate in an academic context can easily be overdone and become counterproductive in the development context. In the absence of a workable consensus on what must be done and how it is to be done, the naysayers tend to carry the day. Political action is much easier to stop than to start. However, in the absence of patient efforts to advance understanding and to build consensus for well-considered programs, sporadic political action to respond quickly—and usually ineffectively—to the latest crisis is also to be expected. In brief, we need to start paying more attention to achieving a consensus and to mobilizing the support required for sustained action to carry good proposals through to effective implementation. The problems that arise because of a multiplicity of proffered options appear to be a particularly significant factor contributing to the lack of progress in reducing malnutrition.

2.4. Cogitation and Interaction

This brings me to the fourth and last of the propositions suggested by a policy analysis perspective. Lindblom (1977) and Wildavsky (1979) have directed attention to "intellectual cogitation" and "social interaction" as two alternative approaches to social problem solving. The cogitation approach presumes that man is up to the task of "thinking through" solutions to problems. The approach of social interaction presumes that we are not. It seeks instead to act out solutions through social processes of voting, markets, bargaining, and the like. Clark and I argue that to deal effectively with ill-structured problems such as development and nutritional improvement requires an integration of cogitation or thinking through approaches and interactive or acting out approaches (Johnston and Clark, 1982, Chapter 1). Hence, we emphasize the need for an approach that recognized the strengths and limitations of both cogitation and interaction.

Good policy analysis includes systematic reflection on the key problems and issues, the major constraints, the feasible opportunities. This reflection and the priorities suggested by analysis of problems, constraints, and opportunities pro-

vide guidance for the design of a variety of program interventions. But our expectations with respect to these interventions should be modest. Shortcomings and mistakes are inevitable. To borrow a phrase from D. N. Michael (1973), "learning to plan" is important, but "planning to learn" is equally important. The challenge is to make good though imperfect choices and then to learn from our inevitable mistakes in a continuing, iterative process of policy design, implementation, and redesign.

3. DESIGNING STRATEGIES FOR ACHIEVING NUTRITION AND HEALTH GOALS

In this section I first highlight certain conditions and constraints that need to be confronted in designing feasible and effective strategies for improving the nutritional status and health of people in low-income developing countries. Because this subset of developing countries share in common a number of important features, it is possible to derive some important implications from an analysis of their economic, structural, and demographic characteristics and associated constraints. In the last two parts of this section, I suggest some priorities with respect to the production-oriented and consumption-oriented programs that should be included in a "multisectoral" strategy.

3.1. Economic, Structural, and Demographic Characteristics of Low-Income Developing Countries

The average per capita GNP of 38 "low-income developing countries" in 1978 was only \$200 compared to an average of \$1250 for 52 "middle-income developing countries." The basic indicators shown in Table I include data for ten selected low-income countries and group averages for the low- and middle-income countries and for 18 industrialized countries.

The basic fact that average incomes are so very low in the low-income countries obviously means that they are subject to severe resource constraints, and the rather slow growth of per capita income in these countries has meant that this economic constraint was being relaxed only gradually. Between 1960 and 1978, the average rate of growth for the low-income countries was less than half the rates of increase in per capita GNP in the middle-income and industrialized countries.

The structural and demographic features of these low-income countries also impose major constraints. Between 1960 and 1978 the percentage of their labor force dependent on agriculture declined only from 77 to 72% compared to a decline from 58 to 45% in the middle-income developing countries and from 17 to a mere 6% in the industrialized countries. The limited structural change in the

Table I
Low-Income Developing Countries: Basic Indicators for Selected Countries and Group Averages^a

Countries	Population (millions) mid-1978	GNP per capita		Percentage of labor force in agriculture	
		U.S. dollars 1978	Average annual growth (percent) 1960-78	1960	1978
Low-income countries ^b					
Ethiopia	31.0	120	1.5	88	81
Bangladesh	84.7	90	-0.4	87	74
Zaire	26.8	210	1.1	83	76
India	643.9	180	1.4	74	74
Pakistan	77.3	230	2.8	61	58
Tanzania	16.9	230	2.7	89	83
Madagascar	8.3	250	-0.3	93	86
Sri Lanka	14.3	190	2.0	56	54
Indonesia	136.0	360	4.1	75	60
Kenya	14.7	330	2.2	86	79
Average for 38 countries (Total population: 1.3 billion)					
		200 ^b	1.6	77	72
Average for 52 middle- income countries (Total population: 873 million)					
		1250 ^b	3.7	58	45
Average for 18 industrialized countries (Total population: 668 million)					
		8070	3.7	17	6

^aData from the World Bank (1980), pp. 110-111, 146-147.

^bThe range is from \$90 to \$360 for the low-income countries and from \$390 to \$3,500 for the middle-income group. The individual countries are listed according to their estimated per capita GNP in 1976 (World Bank, 1978, pp. 76-77).

low-income countries was to large extent an inevitable consequence of two characteristics of their economies: the fact that the agricultural sector still weighs so heavily in their total labor force and the rapid rate of growth of the total labor force. Those two factors plus the rate of increase in nonfarm employment determine the rate of change in a country's agricultural labor force as indicated by the following identity:

$$L'_a \equiv (L'_t - L'_n) \frac{1}{L_a/L_t} + L'_n$$

where L'_t and L'_n are the rates of increase in the agricultural labor force, L'_t and L'_n is the annual percentage rate of increase in the agricultural labor force, L'_n are the rates of increase in the total and nonfarm labor force and L_a/L_t is the initial share of agriculture in the country's total labor force.

The average rate of natural increase in the low-income countries was 2.4% in 1960 and also 2.4% in 1978. This was because the estimated decline in their average crude birth rate from 48 to 39 per thousand was exactly offset by a decline from 24 to 15 per thousand in their death rate (World Bank, 1980, p. 144). There is, of course, a time lag before a change in a country's rate of natural increase is reflected in a change in the rate of growth of its labor force; and a change in a country's labor force participation rate may also affect the rate of change in its labor force. Nevertheless, we can infer that in recent years the rate of growth of the total labor force in the low-income countries has probably been about the same as their rate of natural increase. Furthermore, the rate of growth of their working age population will continue at this high level for some 15 to 20 years before an eventual decline in their rates of natural increase will have a significant effect on labor force growth rates. Thus, the combination of these structural and demographic characteristics means that virtually all of the low-income developing countries will remain predominantly agricultural well into the 21st century.

The decline in the average crude birth rate in the low-income countries between 1969 and 1978 from 48 to 39 per thousand was influenced strongly by rather substantial declines in fertility in India and Indonesia because this is a population-weighted average. The reduction in the crude birth rate as an unweighted average was only from 47 to 45 per thousand, reflecting the fact that most of the low-income countries experienced virtually no reduction in fertility between 1960 and 1978. The crude death rate declined considerably in nearly all of the low-income countries between 1960 and 1978—from 24 to 15 per thousand as a population-weighted average (World Bank, 1980, p. 144). In spite of this decline in overall mortality rates, however, infant and child mortality rates have remained high in most of these countries. It is estimated, for example, that the child death rate (deaths among children aged 1 through 4) declined from 30 to 20 per thousand between 1960 and 1978, but the rate of 20 per thousand was twice as high as that found in the middle-income developing countries and 20 times as high as that found in the industrialized countries (World Bank, 1980, pp. 150–151).*

There is now a wealth of evidence that demonstrates that the persistence of

*Infant mortality figures for developing countries are often underestimated (Vallin, 1976). These estimates of child mortality are only rough approximations, but they give a fair indication of the much greater extent to which mortality has been reduced in the middle-income and industrialized countries as compared to the low-income countries.

unacceptably high infant and child mortality rates in the low-income developing countries is influenced strongly by the two-way interactions between nutritional status and disease and related health factors. The high rates of mortality among infants are in part the result of the poor nutritional status of their mothers and prevalence of low birth weights among newborns. In addition, the poor nutritional status of many infants and small children increases the prevalence and especially the severity of their health problems. Moreover, frequent bouts of dysentery and other episodes of illness frequently precipitate serious malnutrition because of impaired appetite and the common practice of withholding food from sick children. Altered metabolism and the loss of nutrients to intestinal parasites may also be significant contributing factors.

3.2. Implications of the Distinctive Characteristics of the Low-Income Countries

Even this brief summary of conditions and constraints in the low-income developing countries suggests a number of important implications with respect to the design of strategies for development and for achieving nutrition and health goals. The economic constraints that these countries face are so severe that there is usually no possibility of carrying out the large resource transfers that would be required for direct measures such as supplementary feeding programs to reduce the nutritional and other deprivations existing among the large fraction of their populations subject to poverty. To be sure, the poverty of those in the lower-income deciles is exacerbated in many cases by unequal distribution of income. Therefore, as I emphasize later, the "pattern" of development as it affects the opportunity for broad participation in the gains of development is as important as the rate of increase in output. The fact remains, however, that accelerated growth of production of food and a wide range of other products is a necessary if not a sufficient condition for the alleviation of poverty and malnutrition in low-income countries.

The conclusion that I want to stress particularly is the need for development strategies that will be effective in attaining the multiple objectives of (1) achieving self-sustaining, cumulative economic growth, (2) expanding opportunities for productive employment (including self-employment in agriculture, (3) reducing poverty with emphasis on malnutrition and excessive mortality and morbidity, and (4) slowing population growth.

A brief retrospective view of recent developments in India and a prospective view of changes in the structural/demographic situation in Kenya underscore the significance of the constraints and multiple objectives that I have stressed. In spite of the considerable and in some ways impressive efforts to develop the Indian economy, there seems little doubt that the numbers subject to poverty and malnutrition have increased substantially during the past two decades.

Two features of India's experience are of great relevance to the problems facing most of the low-income developing countries. First, recent estimates suggest that in spite of the very considerable industrial growth that has occurred in India, some 74% of the labor force was still dependent on agriculture in 1978, the same share as in 1960 (World Bank, 1980, p. 146). This has been influenced by a considerable emphasis on investments in relatively capital-intensive industries such as steel and chemical fertilizers which has restricted the expansion of other nonfarm employment opportunities. But to a large extent, the lack of structural change was a consequence of the structural and demographic features emphasized above. An important implication of this continuing dominance of agriculture in India's population and labor force is that poverty is to a large extent concentrated in rural areas.

Thus, a second important feature of India's recent experience is the substantial growth in the size of its agricultural population and a consequent decline in the average size of farm holdings—from 6.3 acres in 1953–1954 to 3.8 acres in 1971–1972.* Of particular relevance to the increase in absolute poverty and to the extent of malnutrition is the fact that over the same period the number of marginal holdings of less than 1 acre increased from 15.4 million to 35.6 million households, and their average size declined from 0.24 to 0.14 acre.

Many factors have contributed to this doubling in the number of marginal households. One factor has been an increase in the size of many large ownership units and the tendency for some large landowners to evict their tenants and begin direct cultivation because of the profitability of the new high-yielding varieties and the availability of tractors at relatively cheap prices. Those changes leading to an increasing concentration of land in large units have, however, been more than offset by other forces. There appears to have been considerable subdivision of large holdings along with the large increase in marginal holdings. Between 1953–1954 and 1971–1972, the number of "big" landowners (15 to 50 acres) declined moderately from 4.3 to 4.1 million units, and the share of the area owned by "big" farmers declined from 35 to 31% of the total. Over the same period, the number of "large" holdings (50 acres and above) was reduced from 604 to 350 thousand, and the share of land in these "large" holdings declined sharply from 18 to 8% of the total. The two intermediate groups—"small" and "medium" farmers (with 1 to 5 or 5 to 15 acres)—were able to expand their farm units about as rapidly as the increase in the number of households in each category. As a result, the average small holding remained essentially unchanged at 2.6 acres, and the average ownership unit for medium farmers was reduced

*The estimates by Vyas (1979) on which this discussion is based were derived from various rounds of the National Sample Survey; the breakdown by size groups refers to land holdings owned by households. This Presidential Address by Vyas to the Indian Society of Agricultural Economics also includes an analysis of changes in individual states.

only slightly from 8.5 to 8.3 acres. There has no doubt been some increase in poverty among some of the small farm units, especially those in areas with poor land and inadequate rainfall. The presumption is strong, however, that the greatest concentration of poverty is among the marginal households and that the number of persons subject to poverty and malnutrition has increased roughly in proportion to the doubling of households in that category.

The seriousness of pressure on the land resulting from rural population growth obviously varies a good deal among countries. Rural population pressure has undoubtedly created considerably more acute problems in Bangladesh and in Java than in India. On the other hand, the limits on expanding the area under cultivation have been much less severe in the countries of sub-Saharan Africa. The problems that lie ahead in absorbing a growing labor force into productive employment, however, are probably more serious in Africa than in India. The small size of their fledgling industrial sectors makes it difficult to expand nonfarm employment opportunities sufficiently to absorb a sizeable fraction of the annual increase in the total labor force. In addition, their rates of natural increase are considerably higher, and the prospects for slowing population growth are not promising.

The problems that confront many of the countries of tropical Africa are epitomized by a prospective analysis of the structural/demographic situation in Kenya. Kenya's current rate of natural increase is estimated at approximately 3.9% (Mott and Mott, 1980). Not only is this much higher than India's rate of natural increase of about 2.1%, but reducing fertility will no doubt be considerably more difficult in Kenya because some 80% of the population is still dependent on agriculture, and the traditional attitudes that reinforce the large-family norm appear to be considerably stronger. This contrast appears to apply quite generally in comparing the prospects for fertility decline in Asia versus the countries of sub-Saharan Africa.

Kenya was one of the first African countries to adopt a population policy, but the efforts to promote family planning have not yet had much impact. The development plan for 1979–1983 (Kenya, 1979, p. 130) suggests that sufficient progress has been made in building up an infrastructure for delivering family planning services so that the current plan period will see the beginning of a reduction in the population growth rate. Inasmuch as many of those who will be added to the labor force by the year 2000 have been or will be born before the anticipated increase in family planning will have an appreciable effect, the 118% increase in the "potential labor force" between 1978 and 2000 under the assumption of a declining birthrate is only a little less than the 125% increase to be expected with a constant birthrate. However, the projected increase in the number of children of primary school age would be a relatively manageable 75% with a declining birthrate compared to the 141% increase to be expected with a constant birthrate. There would also be a notable difference in the difficulties that

parents would face in feeding their children and also in the requirements for achieving more adequate coverage in the provision of health services.

The implications of rapid population growth obviously become more dramatic as the time horizon is expanded. Recent projections for Kenya trace the effects on population growth until the year 2024 on the basis of six scenarios of possible changes in fertility and mortality that might occur between 1969 and 1999 (Shah and Willekens, 1978). The most likely estimate, according to these projections by Shah and Willekens, is for a nearly sixfold increase in Kenya's population from 10.9 million in 1969 to 64.3 million in 2024. These projections are broken down by age group and by rural-urban location and therefore have important implications for the problem of absorbing a growing labor force into productive employment. Shah and Willekens (1978, pp. 29, 38) project that the share of the rural work force in the total would decline from 87 to 65% between 1969 and 2024. A sixfold increase in the total population of economically active age would be associated with a 16-fold increase in the active-age population in urban areas, but, nevertheless, there would be a fourfold increase in the rural work force.

In summary, it is clear that the low-income developing countries face formidable problems in attaining the multiple objectives of development. Because of the severity of their resource constraints, it is particularly important for them to confront the issue of "feasibility and desirability" and the fact that there are competitive trade-offs between the goals of increasing productive capacity and output and measures to raise consumption levels more directly. In a somewhat similar vein, Uma Lele (1975, p. 123) has stressed that "a substantial allocation of essential resources to social services frequently occurs at the cost of more immediately productive investments . . . and therefore, may prove self-defeating in the long run." But it is also important to recognize that there are some significant complementarities between some types of production-oriented programs and consumption-oriented activities, especially social services. The complementarity that is most obvious in the light of the earlier discussion of demographic factors is the relationship between the success of production programs aimed at raising per capita output and efforts to slow population growth rates. The discussion of production and consumption programs in the remainder of this section gives considerable attention to such complementarities and to the possibility of designing program interventions so that they will be as effective as possible in furthering multiple objectives.

3.3. Production-Oriented Programs and the "Pattern" of Agricultural Development

In this chapter, I can only briefly consider three propositions related to the design of production programs that are especially pertinent to "multisectoral

nutrition interventions.” (For discussion in greater detail, see Johnston and Kilby, 1975, Chapters 4–6, 9.) These relate to (1) the choice between a “unimodal” or broad-based strategy for agricultural development and a “bimodal” or dualistic strategy, (2) the fundamental importance of the expansion of farm and nonfarm employment opportunities, and (3) the interrelations between the pattern of agricultural development and the prospects for slowing population growth.

3.3.1. Unimodal versus Bimodal Agricultural Strategies

Because of their structural and demographic characteristics, the low-income developing countries face a choice between a unimodal agricultural strategy involving the progressive modernization of the great majority of farm households and a bimodal strategy which emphasizes the “crash modernization” of a sub-sector of atypically large and capital-intensive farm units. Frequently, the choice is made by default. That is, governments favor the expansion of large “modern and efficient” farm enterprises by policies such as an overvalued exchange rate, high protection of imports of consumer goods combined with zero or very low tariffs on tractors, and subsidized credit from institutional sources which mainly goes to the large farm units.

Those policies are often coupled with a failure to support agricultural research and other programs oriented toward the needs of small-scale farmers. The outcome is that the favored large-scale sector accounts for most of the increase in commercialized production and thereby preempts the domestic market. As a result, the bulk of the country’s farmers continue to face a severe cash income and purchasing power constraint. Consequently, they find it extremely difficult to augment their “internal” resources of labor and land with “external,” purchased inputs such as fertilizers, even if national research programs have been successful in developing high-yielding, fertilizer-responsive crop varieties adapted to local conditions.

Given the structural and demographic characteristics of the low-income, late-developing countries, a well-designed unimodal agricultural strategy offers significant economic as well as social advantages. The experience of Japan, Taiwan, and South Korea is especially significant in demonstrating the feasibility of unimodal agricultural strategies that emphasize technical innovations adapted to the needs of small farmers. In the broad view of economic efficiency that is relevant, unimodal strategies are more efficient than bimodal development because they involve gradual expansion in the use of divisible inputs such as fertilizers which complement rather than replace the relatively abundant stock of farm labor which has a very low opportunity cost because of the paucity of nonfarm employment opportunities.

Fortunately, there is now a considerable consensus concerning the feasi-

bility and desirability of emphasizing broadly based, employment-oriented strategies for agricultural development (see, for example, World Bank, 1975, 1978; Asian Development Bank, 1978; India, 1978, Chapter 9; Philippines, 1977, Chapter 5; Kenya, 1979, Chapters 2, 6; Berry and Cline, 1979; Mellor, 1976). There are, however, very difficult problems to be overcome in translating intentions into effective action to achieve a pattern of agricultural development that enables a large and growing fraction of the farm population to benefit from improved income-earning opportunities. This is true even when there is a strong commitment to the goal of fostering broad-based agricultural development.

Unfortunately, the commitment to further broad-based agricultural development is often lacking for a number of reasons. First, there is still a common tendency to assume that the technical superiority of "modern inputs" such as tractors and even combine harvesters means that large-scale, mechanized technologies are also superior in terms of economic efficiency. Second, large farmers and those who identify with them have a vested interest in policies that continue to concentrate scarce resources in a large-scale, highly commercialized subsector. And those groups wield considerable political and economic power. Third, "urban bias," which is such a common phenomenon in developing countries, encourages governments to adopt a "cheap food policy" for urban consumers and other measures that reduce the incentives and resources available for expanding agricultural production (Lipton, 1977). Compensatory measures such as subsidized credit and subsidized inputs are often adopted to counter the adverse effects of low agricultural prices and other policies that have negative effects on farm output. But typically, these compensatory measures benefit only the larger farmers, and the great majority of farmers continue to be affected adversely by the economic and trade policies mentioned earlier.

A number of factors that militate against the successful pursuit of unimodal agricultural strategies are discussed in Johnston and Clark (1982, Chapter 3). These include the commonly recognized factors such as the highly skewed size distribution of farm operational units, the failure to implement land reform legislation, and the inadequate allocation of resources for support services such as agricultural research and for investments in irrigation and other types of infrastructure. Johnson and Clark also argue that inadequate attention to the special problems of increasing farm productivity in regions dependent on rain-fed agriculture and to issues of organizational design and performance has been of major importance.

3.3.2. Expansion of Farm and Nonfarm Employment Opportunities

A unimodal strategy of agricultural development leading to gradual but widespread increases in farm cash incomes also generates a pattern of rural

demand for consumer goods and agricultural inputs which stimulates more rapid growth of nonfarm output and employment than the bimodal alternative. As small farmers participate in widespread increases in productivity and income, their demand for new products is directed toward relatively simple items such as a bicycle or an electric fan and a wider range of improved animal-drawn equipment. In contrast with the automobiles, tractors, and other expensive and sophisticated items demanded by large-scale farmers, these items can be manufactured with satisfactory and increasing efficiency by small- and medium-scale firms using relatively labor-intensive technologies and maximizing the use of domestic raw materials. In contrast, products such as tractors and automobiles are either imported or manufactured by capital- and import-intensive technologies which offer little in the way of expanded employment opportunities.

Thus, in addition to permitting more rapid expansion of opportunities for productive employment within agriculture, a unimodal strategy can be expected to foster more rapid growth of job opportunities in manufacturing and other nonfarm sectors. This is of great importance in relation to achieving the goals of reducing poverty and malnutrition. Expansion of agricultural production will obviously have little impact in reducing malnutrition unless the low-income households with the most serious nutritional problems are able to raise their levels of food consumption. For farm households, increases in productivity and output are needed to permit increased consumption of home-produced foods. For the households of landless laborers and for families in the nonfarm sectors, increased incomes and purchasing power are the indispensable requirement for raising levels of food consumption. Historical experience confirms economic theory in emphasizing that growth in the demand for labor at a rate more rapid than the expansion in the supply of workers seeking employment is the most reliable means of obtaining increased returns to labor. And especially for the low-income households which possess very little in the way of income-earning assets other than their own labor, increases in labor incomes are of crucial importance in reducing poverty and nutritional and other deprivations.

3.3.3. The “Pattern” of Agricultural Development and the Slowing of Population Growth

There is fair amount of evidence that suggests that changes in attitudes and behavior among the farm population induced by a unimodal pattern of agricultural development will create a favorable environment for the spread of family planning. John Stuart Mill advanced this idea many years ago when he argued that an agricultural system based on peasant proprietorship would have a beneficial effect on the “prudence” as well as the “industry” of the farm population and would therefore “discourage an improvident increase in their numbers . . .” (Mill, 1870, concluding paragraph of Chapter 7). More recently, Eva

Mueller's (1971) analysis of the remarkably rapid spread of family planning among the farm population in Taiwan prompted her to argue that the expansion of economic horizons and the rising expectations of Taiwan's farm households as they participated actively in development contributed to the spread of knowledge, attitudes, and motivation essential to the practice of family planning. It is certainly a reasonable hypothesis that conscious action to limit family size will take hold more readily if farm households are actively involved in the modernization process than if they are essentially bypassed by economic progress as under a bimodal strategy.

I have examined elsewhere some of the socioeconomic characteristics of countries that experienced a "significant" reduction in their rate of natural increase between 1960 and 1978 (Johnston and Clark, 1982, Chapter 4). Most of the 12 countries that were identified as having significant reductions in population growth were middle-income countries with per capita GNP well above the levels prevailing in the low-income developing countries.* Broad participation in agricultural development was highly correlated with the slowing of population growth in most of the countries, especially in countries such as China, Taiwan, South Korea, and Malaysia which still had a high percentage of their labor force in agriculture in 1960.†

3.4. Consumption-Oriented Programs: Nutrition, Health, and Family Planning

The recent enthusiasm for a "basic needs approach" to development has focused attention on a variety of programs intended to satisfy basic needs more directly than the income-generating activities I have just considered. An obvious but important implication of the basic needs concept is that some needs are more "basic" than others. This directs attention to the fact that it is important to focus on the composition of the goods and services produced and consumed in a country as well as on the growth and distribution of output.

The consumption-oriented programs that can have direct effects on the composition of goods and services available can be divided into two broad categories—social service programs and measures for the redistribution of current income flows. School lunch programs and schemes for free or subsidized

*"Significant" reductions in fertility were defined as a reduction in the crude birth rate between 1960 and 1978 that exceeded the decline in the country's crude death rate by 6 per thousand or more. Singapore and Hong Kong also met this somewhat arbitrary "test of significance" but were excluded from the analysis because of their predominantly urban populations.

†Malaysia is a special case, however, because substantial development of the small-holder sector of agriculture has been paralleled by an important plantation sector. This parallel development was possible because of the exceptional importance of agriculture exports in Malaysia based initially on rubber and more recently on palm oil as well.

distribution of food are important examples of redistributive measures. Land reform is an even more important example of a redistributive measure. However, redistributive land reform affects the distribution of a productive asset rather than the distribution of current income flows and was therefore considered in the discussion of unimodal versus bimodal strategies of agricultural development.

I focus here on social service programs designed to provide wider access to nutrition, health, and family-planning services. These services partake of the character of "public goods" because they can be provided more efficiently and with greater benefits to society when they are handled as social service programs. To rely on services supplied in response to private demands for these services represents a slow and inefficient approach to promoting improvements in nutrition and health and the spread of family planning.

Education is, of course, another very important social service program. I do not deal with education in this chapter, however; it is not as directly related to promoting nutritional improvement as are health and family planning activities. To be sure, insuring that nutrition and health education are included in the curricula of primary and secondary schools is a potentially important nutrition intervention but one that does not receive attention in this chapter. The importance of education as a public good is widely recognized. Moreover, there is now general acceptance of the proposition that expenditures on education are an investment that contributes to economic growth in addition to conferring benefits on those receiving education (see, for example, World Bank, 1980, pp. 46–53). It is also widely recognized that education, especially of women, contributes to the changes in attitudes and behavior required for the reduction of fertility, and education can contribute to improved nutrition and health, especially if a school's curriculum gives these subjects the attention that they deserve. Most developing countries are, in fact, allocating a substantial fraction of the government budget to education. One recent review of educational problems and issues in developing countries even argues that the expansion of outlays for education may have been too rapid (Blaug, 1979).

The problems of "ineffectiveness and lack of consensus" appear to have had especially serious detrimental effects on progress in promoting nutritional improvement in developing countries. I argue in the final section of this chapter that failure to face up to the trade-offs between redistributive measures such as food subsidies and supplementary feeding programs and action to strengthen social service programs for promoting improvements in nutrition and health have been partly responsible for this lack of progress.

A substantial but insufficient consensus has formed concerning the advantages of integrated programs designed to enable a country's population, rural as well as urban, to have access to a composite package of nutrition, health, and family-planning services (Taylor, 1977; Johnston, 1977; Johnston and Clark, 1982, Chapter 4). The commitment to "primary health care" expressed in the

Declaration of Alma-Ata is a favorable omen (WHO, 1978). It will, however, be extraordinarily difficult to translate the rhetoric of that international resolution into effective national programs.

There is little doubt about the potential advantages of linking nutrition, health, and family planning activities in an integrated "family welfare" or rural health program. Indeed, given the severe budget and manpower constraints that have to be reckoned with in low-income countries, there is probably no other feasible way of achieving broad coverage of the 60 to 80% of their populations that live in rural areas. For that reason, the discussion here focuses mainly on rural health schemes embracing nutritional and family-planning activities as well as health objectives.

There are several reasons why a multisectoral perspective on nutrition interventions suggests that in low-income countries a high priority should be given to integrated health programs capable of achieving broad coverage of the rural population. First, to rely only on income-generating measures that increase total food supplies is an inefficient approach to improving the nutritional status of infants, small children, and their mothers. The nutritional problems of those vulnerable categories are frequently a result of faulty child-feeding practices and failure to recognize the special nutritional requirements of pregnant and lactating women. Even in rural areas, the health of infants is often jeopardized by a shift from breast milk to infant formulas under sanitary and economic conditions that make it exceedingly dangerous to rely on bottle feeding. This is because of the disease problems resulting from contamination and the nutritional effects of excessive dilution of the formula because of insufficient purchasing power.

Second, there are highly important complementarities among nutrition, health, and family planning so that the three sets of activities can be mutually reinforcing if they are combined within a single program. The complementarities related to the two-way interactions between nutritional status and health have already been emphasized. There is also considerable evidence that suggests that reducing infant and child mortality to "acceptable" levels and promoting increased awareness that the risk of child loss has been reduced substantially can increase the "demand" for family planning services. Thus, a rural health program that embraces family planning and achieves broad coverage can reduce the psychic as well as the objective costs of fertility regulation. These complementarities together with certain possibilities for reducing costs within an integrated program as compared to single-service programs means that a composite package approach can be more cost-effective in realizing the interrelated objectives of improving nutritional status and health and slowing population growth.

Third, there is at least the possibility of mobilizing a stronger support coalition to insure sufficient political, financial, and administrative support for health programs capable of achieving broad coverage because of the multiple objectives that are being promoted. Fourth, there is now convincing evidence

that health programs that emphasize preventive and promotive activities, including nutrition education, hygiene, and an immunization program, can be very effective in reducing infant, child, and maternal mortality and at a cost that is affordable even in the low-income developing countries.

Although the potential advantages of integrated nutrition, health, and family-planning programs are impressive, it must be emphasized that the design and implementation of programs capable of realizing that potential is an exceedingly formidable undertaking. It is clear from demonstration projects in India and a number of other countries that an effective methodology is available (Gwatkin *et al.*, 1979; Pyle, 1979). Experience in China (and perhaps Costa Rica and Sri Lanka) demonstrates that programs capable of achieving nationwide coverage are not impossible. But the problems to be overcome in moving from small demonstration projects to the implementation of national programs are enormous (Pyle, 1981).

Reasonably effective implementation of such programs will require a sustained effort and a willingness to learn from both successes and failures in order to progressively increase their efficacy. That is only one of the reasons why the range of services and geographical coverage should be expanded gradually in a sequential fashion. Hard decisions must be made with respect to the objectives and components of rural health programs. As already suggested, it seems important to give priority in the initial phase to infants and small children and their mothers. That priority is justified in the first instance by the disproportionate concentration of mortality and morbidity among these groups and by the significant long-term benefits of avoiding the nutrition problems that impair the physical and cognitive development of children. In addition, the interventions available for direct action to reduce nutrition and health problems within these groups are much more cost-effective than programs aimed at older children and adults. It can be argued that nutrition problems among the productive members of a household represent an even more serious problem, but the most realistic approach to resolving those problems is through raising incomes and food consumption levels by the broadly based, employment-oriented development strategies discussed earlier.

There is continuing debate over the effectiveness and even the feasibility of integrated health programs. It has been suggested that "integrated programs require stronger management to maintain the same level of performance as a comparable vertical program" and that community-based health programs that emphasize preventive and promotive activities involve an "internal contradiction" which makes it doubtful whether national programs can replicate the success of small demonstration projects (Korten, 1975, pp. 25, 26). In spite of international policy statements such as the Declaration of Alma-Ata, there is strong resistance among the medical profession to a health strategy that differs so drastically from the "Western tradition" of individual curative care. It is re-

ported that even in China, the "barefoot doctor" program required strong personal intervention by Chairman Mao to counter the biases of physicians toward curative care and maintaining standards rather than achieving broad coverage (Maru, 1977). Moreover, community-oriented programs require skills that are not developed in conventional medical training programs. A critical role of physicians in these programs is to provide training and supportive supervision for auxiliaries and community health workers. Furthermore, the behavioral changes that are required to achieve significant and lasting benefits require a "partnership" between the health team and the local communities. A need for "local participation" is easy to assert but difficult to achieve. It requires an investment of time and effort by members of the local community which will be forthcoming only if the benefits are perceived as being sufficiently attractive to justify that investment.

Many nutrition and population specialists are either opposed to integrated programs or provide only grudging support. It is reported, for example, that "many population specialists take the view that fertility limitation is too urgent a task to leave to health organizations. . ." (Finkle and Crane, 1976, p. 368). In analogous fashion, many nutrition specialists are concerned that the medical personnel responsible for an integrated program will have little appreciation of the fundamental importance of nutrition and of the significance of the interactions between nutritional status and infection.

Although the resistance of population and nutrition specialists can be attributed in part to a normal bureaucratic preference for emphasizing one's own particular program, there are more legitimate reasons for concern. Preventive and promotive activities are more difficult than the passive role of, for example, dispensing a few drugs, so there is a real danger that they will be neglected. In the early phases of implementing India's Community Health Worker Program, for example, there was a tendency for village health workers and health personnel at higher levels to emphasize individual curative care rather than preventive and promotive activities (Pyle, 1981). Moreover, local people as well as medical personnel are likely to be more concerned with curative care. It is reported, however, that active participation of local groups such as village health committees promotes a community orientation and greater recognition of the fact that preventive and promotive activities are most cost-effective.

The experience with demonstration projects suggests that giving a high priority to the use of simple nutrition screening procedures can make a notable contribution to increasing the effectiveness of rural health programs. Maintaining weight-for-age charts or, if scales are too costly, mid-upper-arm circumference measurements makes it possible to identify all infants and small children in a village or other local community with moderate or serious malnutrition. This information can increase the effectiveness of nutrition education and also enhance the prospect that external and especially local resources will be used to

correct the deficiencies that are identified. Robert Chamber (1977, p. 2) has aptly observed that the poor rural households, which contain a disproportionate share of the malnourished, are “relatively invisible.” Nutrition screening at the local level appears to be an especially promising means of overcoming that problem and of facilitating more focused corrective action.

4. TOWARD A WORKABLE CONSENSUS AND EFFECTIVE ACTION

The principal conclusion put forward in the preceding section is that a very high priority should be given to simultaneous and parallel efforts to promote broadly based agricultural development and to provide wide access to nutrition, health, and family-planning services. It was also emphasized that in the design and execution of those two “prongs” of a two-pronged strategy, explicit attention should be given to attaining four interrelated objectives: accelerating the growth of output, expanding employment opportunities, improving nutrition and health, and slowing population growth.

There are, of course, many obstacles to the success of such a two-pronged strategy. It seems to me that as “policy analysts” concerned with achieving more effective action to reduce malnutrition and related deprivations, we need to give particular attention to two sets of problems. First, we need to give particular attention to achieving a workable consensus and mobilizing a support coalition for the programs that will be feasible and most effective in achieving the development objectives that merit priority, objectives that include but cannot be confined to the goal of improved nutrition. Second, we should recognize that learning how to implement programs is as important as determining what to do and that this will require much more explicit attention to issues of organization and management.

4.1. Mobilizing a Support Coalition

Under the best of circumstances, effective action to eliminate malnutrition and related deprivations in the low-income developing countries will be difficult because of bureaucratic inertia, the opposition of vested interests, and the sheer magnitude of the task and the scarcity of resources. It is precisely because of those difficulties that widespread and sustained progress requires that only a few of the highest-priority and most mutually reinforcing programs should be undertaken. And that means choosing not to do many things that appear to be attractive in a short-run, partial view.

Because of their awareness of the seriousness of nutritional problems, nutrition advocates have an understandable interest in finding short-cut solutions and

an equally understandable impatience with development strategies which have often failed to reduce malnutrition and related manifestations of poverty. And they have frequently supported school lunch programs and other supplementary feeding schemes, food subsidies, and proposals for food stamp plans or "food entitlement schemes," no doubt because of a desire to produce immediate results and to do what can be done. Those are commendable attitudes, and a prolonged search for the "best" strategy can be an obstacle to the progress that is possible. But given the severe resource constraints in poor countries, the opportunity cost of devoting attention and resources to short-run welfare measures is enormous. Moreover, the influence of donor agencies and foreign advisors in advocating short-run welfare measures is likely to be especially significant and unfortunate because they reinforce and legitimize the domestic political pressures that encourage an "urban bias" and a preoccupation with immediate, short-term concerns.

Typically, recommendations to undertake costly programs such as a food subsidy program fail to consider their opportunity cost. An example is the endorsement of food stamp programs in the Report of the Presidential Commission on Hunger (1980, pp. 40–41) and the advocacy of similar programs by the World Food Council (1980) under the rubric of "food entitlement schemes." An endorsement of food subsidy programs by Gavan (1979) is somewhat unusual in that he recognizes explicitly that "foreign assistance must meet a major part of the burden" and that "funds to help maintain farm prices must be forthcoming as well." I believe, however, that it is unrealistic and irresponsible to assert that large increases in foreign aid are likely to be forthcoming. Experience to date suggests that earmarking of foreign aid for consumption programs tends to be at the expense of more fundamental programs such as investments in infrastructure and in institution building.

Although some deprivations of certain target groups can be alleviated by short-run welfare measures, preoccupation with such measures will almost certainly divert resources, attention, and support away from more fundamental measures. The pervasive problems of poverty, especially among the hard-to-reach rural population, are likely to be accentuated. Indeed, that is one of the most important implications of the analysis in Section 3 of the characteristics of the low-income developing countries and of the major constraints and opportunities they face: the problems of rural poverty will become more widespread and more intractable unless actions are focused on programs to strengthen indigenous capacities to accelerate the growth of output, to expand income-earning opportunities within and outside agriculture, and to strengthen the most essential social services.

In fact, some development specialists argue that the task of achieving broad-based agricultural development is so demanding and so important that social service programs should be deferred. As noted earlier, Lele (1975, p. 123) has

argued that substantial allocation of scarce resources to social services is frequently at the cost of more immediately productive investments in rural areas “and therefore, may prove self-defeating in the long run.” I have argued, however, that we need to recognize the complementarity as well as the competitive trade-offs between production- and consumption-oriented programs: the challenge is to achieve an “appropriate” balance in the allocation of scarce resources between those prongs of a two-pronged strategy. In particular, I have suggested that failure to deal effectively with the interrelated objectives of improving nutritional status and health and promoting the spread of family planning is also likely to be self-defeating in the long run.

The emphasis that I have given to integrated nutrition, health, and family-planning programs is bound to be controversial because of the limited evidence concerning the feasibility of an integrated approach. There is no doubt that the design and effective implementation of programs to provide a “composite package” of nutrition, health, and family-planning services poses formidable organizational and management problems. After reviewing “the imposing arguments for integration,” Watson *et al.* (1979, pp. 160–162) conclude that: “The ultimate answers will derive from empirical experience rather than eloquent arguments or counterarguments.” But the lessons to be derived from experience with integrated nutrition, health, and family-planning programs will depend to a large extent on whether explicit and sustained attention is given to problems of organization and management.

4.2. Organization and Management as a Third “Prong” in Development Strategies

Questions of organizational design and managerial procedures have received remarkably little attention in the planning and implementation of development. But clearly, effective development requires organization. Brewster (1967, p. 69) has described this as “the capacity for concerting reciprocally helpful behaviors into a continually widening network of specialized units of collective action. . . .” These issues of organization are especially critical in providing “public goods” that will not be available at socially optimal levels without interventions by publicly supported organizations. I have emphasized that social service programs for providing broad access to nutrition, health, and family planning partake of the character of public goods and therefore merit a high priority among government programs. Similarly, success in fostering widespread increases in productivity and output among small-scale farmers is very dependent on effective support institutions for agricultural research, extension, and for the construction and management of irrigation facilities and other types of infrastructure. The East Asian countries of Japan, Taiwan, South Korea, and the People’s Republic of China are notable for the attention that has been given to creating a

network of organizations capable of providing those support services. Their success in enabling their rural populations to benefit from effective health and family planning programs has also depended on the attention that has been given to questions of organization and management.

The more usual neglect of organizational issues in the development literature and in practice presumably reflects the view that organizational choices are unimportant or that they are not a subject that governments can do something about. In fact, they are subject to experimentation and government action. To be effective, however, government efforts to influence the design and management of development organizations must be guided by an understanding of the constraints and opportunities associated with particular organizational choices.

Important and rather different issues arise in connection with two types of organizations: (1) local level organizations involving "community participation" and (2) "facilitator organizations" for linking people at the local level with a development bureaucracy responsible for providing public goods such as research, agricultural extension, and nutrition and health services. The success of rural development efforts depends not only on the design and performance of individual organizations but also on the establishment of effective vertical and horizontal linkages to achieve a good "fit" among (1) the competence of various organizations, (2) the programs that are being implemented, and (3) the needs of the people in rural communities who are the intended beneficiaries (Korten, 1980).

William Clark and I have recently examined those two sets of problems in considerable detail because we are persuaded that organization programs demand serious and sustained attention and should be regarded as a third "prong" of a three-pronged strategy for rural development (Johnston and Clark, 1982, Chapter 5).^{*} Organization is important in part because attempts to change what things are done must be accompanied by changes in how things are done. It is useful to view organization as providing a framework for "calculation and control" through which collections of individuals determine what each is to do and seek to insure that each does what is expected of him (Dahl and Lindblom, 1953). The techniques of calculation and control include the informal face-to-face bargaining that is so important in local community organizations, the hierarchical techniques that characterize "facilitator organizations" and other government bureaucracies, and exchange techniques of which price and market mechanisms are an especially significant example.

There is a growing body of evidence that demonstrates that a "blueprint approach," which views organizations essentially as a means of assisting planners and administrators at higher levels in implementing their plans, is not an

^{*}Although this book is a collaborative enterprise, Clark was mainly responsible for the chapter on organization programs. This brief account does not do justice to his much more extended treatment.

effective approach. Rather, there is a need for a "learning approach" that promotes a mutual learning process involving local people, field staff, and specialists in addition to the higher-level administrators in facilitator organizations.

Local participation and community organizations such as village health committees appear to be especially important in the design and implementation of nutrition, health, and family-planning programs. The success of such programs depends on changes in behavior and in community norms, precisely the sort of changes that are facilitated by active local participation. It has become fashionable in recent years to emphasize the importance of local participation, but unfortunately, most of the discussion has not gone beyond the level of rhetoric. There is, for example, a tendency to treat participation as a free good. In fact, participation in a local organization requires an investment of time and energy by the participants. And that investment cannot be commanded by administrators, it must be induced. The ability to attract effective participation appears to depend mainly on three features of local organizations: (1) the attractiveness of the benefits, (2) a relative harmony of objectives, and (3) simplicity of technique. Local groups such as a village health committee set up to support the work of a village health worker and a community nutrition, health, and family-planning program appear to rank high on all three counts. This does not mean that the task of promoting such local organizations is an easy one. It does mean that it is feasible and merits serious attention because of the great importance of active local participation in attaining the potential benefits of an integrated nutrition, health, and family-planning program.

Organizational issues and the need for an adaptive, sequential learning process of policy design, implementation, and redesign also apply to the task of designing and implementing broad-based strategies for agricultural development. However, the need for serious and sustained attention to these questions appears to be especially great in relation to integrated nutrition, health, and family-planning programs. As was previously noted, there is very limited experience in coping with the problems of moving from small-scale demonstration projects to effective nationwide programs. It is not at all surprising, for example, that serious problems are being encountered in India in implementing the Community Health Worker Program that was launched in 1977. And the difficulties that are being encountered in securing professional legitimization of a health strategy emphasizing preventive and promotive activities because of the opposition of many physicians were certainly predictable.

Cogitation alone cannot possibly provide answers to the difficult problems that must be overcome in evolving effective community-oriented health programs. Analysis of the characteristics of the low-income countries is important in emphasizing the potential contribution of an integrated approach. Such analysis also underscores the difficulty, if not impossibility, of identifying alternative means of furthering the interrelated objectives of improving nutritional status and

health and slowing population growth that will be feasible and effective in a low-income developing country. But an interactive “acting out” approach and applied research are needed in order to learn from the successes and failures of the ongoing experience in India and other countries.* There also appears to be an important need for comparative studies to facilitate learning as much as possible about key problems such as fostering effective community participation, sustaining the motivation of village-level workers, and devising methods of financing that include contributions from the local community and private payments to cover the cost of drugs except in the case of the poorest families. This action-oriented research calls for a genuinely collaborative effort by scientists, policy-makers, and administrators. Sustained efforts in research and in pursuing an adaptive approach to the problems of management are not likely to be realized, however, without the support of nutritionists, health scientists, and population specialists. It is for that reason that it is so important for the scientific community in those disciplines to go beyond their specialties and to play their role as good policy analysts in order to be effective.

REFERENCES

- Asian Development Bank, 1978, *Rural Asia: Challenge and Opportunity*, Praeger, New York.
- Berg, A., 1973, *The Nutrition Factor*, The Brookings Institution, Washington.
- Berg, A., Scrimshaw, N. S., and Call, D. L. (eds.), 1973, *Nutrition, National Development and Planning*, MIT Press, Cambridge, Massachusetts.
- Berry, R. A., and Cline, W. R., 1979, *Agrarian Structure and Productivity in Developing Countries*, Johns Hopkins University Press, Baltimore.
- Blaug, M., 1979, The quality of population in developing countries with particular reference to education and training, in: *World Population and Development: Challenges and Prospects* (P. M. Hauser, ed.), Syracuse University Press, Syracuse, pp. 361–402.
- Bose, A., Goyal, R. P., Grover, S. R., Jolly, K. G., Madan, D., Sharma, V. P. C., and Singh, C., 1978, *An Assessment of the New Rural Health Scheme and Suggestions for Improvement*, Demographic research Centre, Institute of Economic Growth, University of Delhi, Delhi.
- Brewster, J. M., 1967, Traditional social structures as barriers to change, in: *Agricultural Development and Economic Growth* (H. M. Southworth and B. F. Johnston, eds.), Cornell University Press, Ithaca, New York, pp. 66–98.
- Chambers, R., 1977, Simple is practical: Approaches and realities for project selection for poverty-focused rural development. Paper prepared for a Seminar on the Implications of the Employment and Income Distribution Objectives for Project Appraisal and Identification, Kuwait,

*The Government of India initiated a program of evaluation shortly after the initiation of the Rural Health Scheme and Community Health Worker Programme in 1977 (see, for example, Bose *et al.*, 1978). The Regional Office for South East Asia of WHO has initiated “an action research programme in nutrition.” A Workshop and a Study-Group Meeting, both held in 1979, outlined a series of studies on topics such as the content and implementation of the nutrition component of Primary Health Care programs and problems related to the promotion of low-cost weaning foods in rural communities (South East Asia Regional Office, World Health Organization, 1979).

- 5–6 April. Available from the Institute of Development Studies, University of Sussex, Brighton, England.
- Dahl, R. A., and Lindblom, C. E., 1953, *Politics, Economics, and Welfare*, Harper & Brothers, New York.
- Field, J. O., 1977, The soft underbelly of applied knowledge: Conceptual and operational problems in nutrition planning, *Food Policy* 2(3):228–239.
- Finkel, J. L., and Crane, B. B., 1976, The World Health Organization and the population issue: Organizational values in the U.N., *Pop. Dev. Rev.* 2(3/4):367–393.
- Gavan, J. D., 1979, Commentary: Food subsidies—imperfect but expedient, *International Food Policy Research Institute Report* 1(2):September 1.
- Gwatkin, D. R., Wilcox, J. R., Wray, J. D., 1979, Can Interventions Make a Difference? The Policy Implications of Field Experiment Experience, A Report to the World Bank, Overseas Development Council, Washington.
- Hirschman, A. O., 1971, *A Bias for Hope: Essays on Development and Latin America*, Yale University Press, New Haven.
- Huenemann, R. L., 1981, Leadership and quality in nutritional care: Our role in today's world, *J. Am. Diet. Assoc.* 78(2):124–128.
- India, Government of, 1978, *Draft Five-Year Plan 1978–83*, Planning Commission, New Delhi.
- Johnston, B. F., 1949, Japan: The race between food and population, *J. Farm Econ.* 31(2): 276–292.
- Johnston, B. F., 1977, Food, health, and population in development, *J. Econ. Lit.* 15(3): 879–907.
- Johnston, B. F., 1982, Integrated multisectoral nutrition interventions at the village level, in: *Nutrition Policy Implementation: Issues and Experience* (N. S. Scrimshaw and M. B. Wallerstein, eds.), Plenum Press, New York, pp. 173–207.
- Johnston, B. F., and Clark, W. C., 1979, Food, Health, and Population: Policy Analysis and Development Priorities in Low-Income Countries, WP-79-52, International Institute for Applied Systems Analysis, Laxenburg, Austria.
- Johnston, B. F., and Clark, W. C., 1982, *Redesigning Rural Development: A Strategic Perspective*, Johns Hopkins University Press, Baltimore.
- Johnston, B. F., and Kilby, P., 1975, *Agriculture and Structural Transformation: Economic Strategies in Late-Developing Countries*, Oxford University Press, New York.
- Joy, J. L., 1973, Food and nutrition planning, *J. Agric. Econ.* 24(1): 165–192.
- Kenya, Republic of, 1979, *Development Plan for the Period 1979 to 1983*, Nairobi.
- Korten, D. C., 1975, Integrated Approaches to Family Planning Services Delivery, Development Discussion Paper No. 10, Harvard Institute for International Development, Cambridge, Massachusetts.
- Korten, D. C., 1980, Community organization and rural development: A learning process approach, *Pub. Admin. Rev.* 40(5) 480–511.
- Lele, U., 1975, *The Design of Rural Development: Lessons from Africa*, Johns Hopkins University Press, Baltimore.
- Leys, C., 1971, Political perspectives, in: *Development in a Divided World* (D. Seers and L. Joy, eds.), Penguin Books, Middlesex, England, pp. 106–138.
- Lindblom, C. E., 1977, *Politics and Markets*, Basic Books, New York.
- Lipton, M., 1977, *Why Poor People Stay Poor: A Study of Urban Bias in World Development*, Temple Smith, London.
- Maru, R. M., 1977, Health manpower strategies for rural health services in India and China: 1949–1975, *Soc. Sci. Med.* 11:535–547.
- Mellor, J. W., 1976, *The New Economics of Growth: A Strategy for India and the Developing World*, Cornell University Press, Ithaca, New York.

- Michael, D. N., 1973, *On Learning to Plan—and Planning to Learn*, Jossey-Bass Publishers, San Francisco.
- Mill, J. S., 1870, *Principles of Political Economy*, 5th London ed., Book 2, New York.
- Morgan, M. G., 1978, Bad science and good policy analysis, *Science* 201:971.
- Mott, F. L., and Mott, S. H., 1980, Kenya's record population growth: A dilemma of development, *Pop. Bull.* 35(3):3–43.
- Mueller, E., 1971, *Agricultural Change and Fertility Change: The Case of Taiwan*, University of Michigan, Ann Arbor.
- Philippines, Republic of, 1977, *Five-Year Philippine Development Plan, 1978–1982*, Manila.
- Presidential Commission on World Hunger, 1980, *Overcoming World Hunger: The Challenge Ahead*, U.S. Government Printing Office, Washington.
- Pyle, D. F., 1979, *Voluntary Agency-Managed Projects Delivering an Integrated Package of Health, Nutrition, and Population Services: The Maharashtra Experience*, prepared for The Ford Foundation, New Delhi.
- Pyle, D. F., 1981, From project to program: The study of the scaling-up implementation process of a community-level integrated health, nutrition, population intervention in Maharashtra (India), Ph. D. Dissertation, Massachusetts Institute of Technology, Cambridge.
- Shah, M. M., and Willekens, F., 1978, *Rural–Urban Population Projections for Kenya and Implications for Development*, International Institute for Applied Systems Analysis, Laxenburg, Austria.
- South East Asia Regional Office, World Health Organization, 1979, *An Action Research Programme in Nutrition for Developing Countries*, WHO South-East Asia Region, New Delhi.
- Stokey, E., and Zeckhauser, R., 1978, *A Primer for Policy Analysis*, W. W. Norton, New York.
- Taylor, C. E., 1977, Nutrition and population in health sector planning, *Food Res. Inst. Stud.* 16(2):77–90.
- United Nations, 1975, *Report of the World Food Conference, Rome, 15–16 November, 1974*, The United Nations, New York.
- Vallin, J., 1976, World trends in infant mortality since 1950, *World Health Statistics Report* 29(11), World Health Organization, Geneva.
- Vyas, V. S., 1979, Some aspects of structural change in Indian agriculture, *Indian J. Agric. Econ.* 34(1):1–18.
- Watson, W., Rosenfield, A., Viravaidya, M., and Chanawongse, K., 1979, Health, population, and nutrition: Interrelations, problems, and possible solutions, in: *World Population and Development* (P. M. Hauser, ed.), Syracuse University Press, Syracuse, pp. 145–173.
- Wildavsky, A., 1979, *Speaking Truth to Power: The Art and Craft of Policy Analysis*, Little, Brown, Boston.
- World Bank, 1975, *The Assault on World Poverty: Problems of Rural Development, Education and Health*, Johns Hopkins University Press, Baltimore.
- World Bank, 1978, *World Development Report 1978*, World Bank, Washington.
- World Bank, 1980, *World Development Report 1980*, Oxford University Press, New York.
- World Food Council, 1980, Toward the Eradication of Hunger: Food-Subsidy and Direct-Distribution Programmes, WFC/1980/3, Report by the Executive Director, Rome.
- World Health Organization, 1978, *Primary Health Care: Report of the International Conference on Primary Health Care, Alma-Ata, USSR, 6–12 September 1978*, WHO/UNICEF, Geneva.

Burkitt's Lymphoma

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1. INTRODUCTION

The occurrence of a malignant tumor that is seen relatively frequently in African children was first highlighted as a clinicopathological entity by Burkitt (1958, 1962a) about two decades ago. Children with such tumors were familiar to some medical workers in tropical Africa and have been documented in hospital records since the early decades of this century (Burkitt, 1962a). Case reports of the peculiar jaw manifestation of the disease were even published by a few doctors before the epoch-making report of Burkitt (1958). The tumor was characterized as a malignant lymphoma by O'Connor and Davies (1960) following which the eponymous title of Burkitt's lymphoma was generally adopted (Berard *et al.*, 1969).

Diverse aspects of the disease have been extensively studied in many institutions situated in geographical areas where patients are seen either frequently or sporadically. Much of the impetus for the studies arose from peculiarities of the disease which revealed very striking pathological, epidemiologic, microbiological, and immunologic features. It is remarkable that although it is a recently appreciated disease entity, studies carried out on the tumor have already yielded deep insights into some of its etiologic factors as well as its pathogenesis and treatment.

Knowledge about Burkitt's lymphoma (BL) and related syndromes has accumulated so rapidly in the past two decades that a comprehensive review of the relevant literature of all aspects of the disease is beyond the scope of this book. It is therefore intended in this chapter to (1) summarize the undisputed general features of the lymphoma, (2) emphasize some clinicopathological fea-

tures that are not generally appreciated at present, and (3) highlight recent advances in knowledge of the lymphoma and related proliferative disorders.

2. GENERAL FEATURES OF THE DISEASE

Over the years some features of Burkitt's lymphoma have emerged from the observations and published reports of several workers as definite and non-controversial. Such features are now generally known to medical and other scientists interested in tropical diseases in general and in childhood lymphomas in particular. This section briefly outlines a review of such clinical, pathological, and epidemiologic features of the lymphoma.

2.1. Clinical Presentation

The main reason why Burkitt's lymphoma was not recognized as a disease entity until the late 1950s is largely that the disease manifests clinically in diverse ways, especially in tropical countries where medical centers are relatively few and acutely ill children are overwhelmingly many. The medical community is, of course, now aware of this relatively common childhood cancer, and the suspicion index for its provisional clinical diagnosis is high everywhere. The disease mainly affects children of school age, although younger children and adults are not exempt (Section 2.1.4). Most patients with the lymphoma usually present with one or more of the following features: (1) facial tumor (2) abdominal swelling, and/or (3) neurological deficit, especially in advanced cases.

2.1.1. Facial Tumor

The most striking and peculiar clinical feature of Burkitt's lymphoma (BL) is the frequency with which the malignant neoplasia affects the facial bones, especially the jaws. Indeed, the disease was initially recognized because of this predilection. Prospective clinical studies on large numbers of cases in East and West Africa have shown jaw swelling to be the most frequent presenting symptom, especially in younger children (Burkitt, 1962a; Clifford, 1967; Ngu, 1968; Nkrumah and Perkins, 1976; Biggar *et al.*, 1979).

The upper jaw is more frequently involved, although patients may present with one, more, or all four quadrants of the jaw. Loosening or loss of the teeth of an affected jaw quadrant is usually manifest clinically and radiologically. Tumors of the jaws and other facial bones arise from the bone marrow and extend in some cases to involve soft tissues of the orbit, giving rise to proptosis, and occasionally to the meninges, brain, and salivary glands.

2.1.2. Abdominal Swelling

Children with BL frequently present with nontender palpable masses in the abdomen with or without clinical ascites (Booth *et al.*, 1961; Ngu, 1967; Ajayi, 1971; Al-Attar *et al.*, 1979; Biggar *et al.*, 1979). The extent of involvement of the various abdominal organs is difficult to ascertain clinically, and this is perhaps the main reason why this feature of the disease has not received the emphasis it deserves. There may be hepatomegaly, and the spleen may be palpable. Girls usually have palpable masses in the lower abdomen suggestive of ovarian tumors. X-ray of the abdomen may show paravertebral tumor shadow. Rarely, cases have presented with symptoms and signs indicative of peritonitis from perforated tumor involvement of the stomach or intestine.

Abdominal tumors may or may not be associated with obvious jaw tumor in both sexes. In girls, the clinical frequency of abdominal tumors is higher than involvement of the jaws, although the reverse is true for boys. This is largely because of the frequent involvement of the ovaries, a feature of BL that was recognized early (Brew and Jackson, 1960).

2.1.3. Neurological Manifestations

Because it is a lymphoma, another peculiar feature of BL is the frequent occurrence of neurological deficits and cerebrospinal fluid pleocytosis in patients with the disease. Flaccid paraplegia with weakness of the lower limbs is the most striking and readily detectable clinical presentation of neurological involvement of the disease. The feature was highlighted by early workers (Janota, 1966; Odeku and Osuntokun, 1968) and led to detailed studies on this aspect of the disease by several other groups of workers (Ziegler *et al.*, 1970a; Nkrumah and Perkins, 1973). Involvement of the central nervous system is usually a late manifestation of the disease and of poor prognostic significance.

Proptosis, which is usually unilateral and caused by tumors in retroorbital tissues, is also a common clinical feature of the disease. Occasionally, it involves both eyes, and in extreme cases there may be destruction of affected eyeball. It is significant to note from the recent prospective clinical studies of Biggar *et al.* (1979) that all cases of BL with evidence of central nervous system involvement had clinically obvious facial and/or abdominal tumor.

2.1.4. Other Clinical Features

The three common features of BL outlined in the preceding sections (facial tumors, abdominal tumors, and CNS involvement) may be present in the individual patient in all possible combinations. Indeed, involvement of multiple organs in each patient is one of the hallmarks of the disease. Apart from clinical

presentation at multiple sites, the following other features of the disease have been established and reported by various workers.

Primary involvement of lymphoid tissues by BL is remarkably minimal, in contrast with other lymphomas. In particular, enlargement of superficial lymph nodes, tonsils, and thymus is not clinically manifested in most patients. Mild to moderate splenomegaly and cervical lymph node enlargement are, however, clinically apparent in many patients. Moreover, even such enlargement is caused by primary lymphoma in only a minority of cases of BL, as revealed by microscopy of biopsy or autopsy material and explained in Section 2.3. On the other hand, the bone marrow of affected bones is a common site of the disease. Bone pains caused by diffuse bone marrow involvement have been described as the clinical presenting feature of some cases (Ngu, 1967; Pike *et al.*, 1967). Similarly, a leukemic picture has been described in a few terminal cases (Wright and Pike, 1969).

Radiological investigations have established that the lymphoma is osteolytic in affected bones and that destruction of dental lamina dura in involved jaws is an early characteristic feature (Cockshott, 1970). Such osteolytic lesions are evident in cases in which there is involvement of bones of the skull vault, long bones of the limbs, and bones of the pelvis and thoracic cage. The demonstration of bilateral paravertebral soft tissue mass in relation to the lower thoracic and upper lumbar vertebral bodies is a distinct radiological feature of cases with paraplegia. Intravenous pyelogram frequently reveals distorted calyces as evidence of tumor involvement of the kidneys in cases presenting with abdominal masses. Recent reports have also indicated the potential usefulness of ultrasound diagnosis in the clinical detection and assessment of the site of retroperitoneal tumor mass (Shawker *et al.*, 1979).

Clinically obvious BL involvement of the thyroid, testis, and breast (Bannerman, 1966; Durodola, 1976; Al-Attar *et al.*, 1979) is generally accepted as a definite feature of the disease. Bilateral involvement is the rule, although a few exceptions have been noted.

As mentioned by Osunkoya and Ajayi (1972), BL patients do not normally show evidence of malnutrition. Their nutritional state is generally comparable to that of healthy individuals of the same age in the community, although cachexia may supervene in patients with late-stage disease or protracted illness.

There are very few reports of BL patients with clinically detectable superimposed infection or concomitant disease. Spectacular spontaneous tumor regression was reported in one case following measles (Bluming and Ziegler, 1971). Clinical complications such as perforated gastrointestinal ulcer, renal failure, or obstructive jaundice are related to anatomic location of tumors or effects of therapy.

Studies from high- and low-incidence areas have established that BL is essentially a disease of childhood, over 90% of cases falling between 2 and 15

years of age (Burkitt, 1962a, Ngu, 1968; Nkrumah and Perkins, 1973; Al-Attar *et al.*, 1979; Biggar *et al.*, 1979). In general, boys are affected more frequently than girls in an overall ratio of about 2:1. The disease is very rarely seen in children aged below 2 years, and patients above 15 years vary widely in proportion from series to series.

2.1.5. Differential Diagnosis

General awareness of the features of BL has increased over the years in areas of both high and low incidence. Clinical suspicion and diagnosis of the disease is usually easy, requiring only confirmatory histopathology or cytology in most cases to exclude diseases such as other childhood tumors, abdominal tuberculosis, poliomyelitis, and sickle cell disease in cases with bone pains and no obvious tumor (Ngu, 1967; Osunkoya and Ajayi, 1972; Biggar *et al.*, 1979).

Contemporary difficulties with the diagnosis of BL relate to controversy over acceptance of the disease as a clinicopathological entity distinguishable from other lymphoblastic lymphomas. Does more than one disease entity produce a lymphomatous tumor with a centroblastic histological picture? This is largely a histopathological problem which is discussed in Section 2.2.

2.1.6. Prognosis

In general, the disease manifests as a highly malignant neoplasia. From most patients' histories, apparent onset of disease is usually a matter of a few weeks before presentation at a medical center. Although a few cases of apparent spontaneous regression of tumor have been reported, the disease is believed to be fatal within 6 months if untreated.

The extent and site of the lymphoma are of prognostic significance in individual patients (Odeku and Osuntokun, 1968; Osunkoya and Ajayi, 1972; Ziegler *et al.*, 1972; Nkrumah and Perkins, 1976). Presentation with only a single facial tumor has the best prognosis for effective treatment. In contrast, widely disseminated tumor and extension of tumor cells into the CNS have the worst prognosis. These generally accepted prognostic features of the lymphoma have led to a large degree of agreement among groups of workers in the clinical staging of the disease.

2.2. Pathology

The main characteristic features of BL are generally known through the large number of published accounts of its clinical, epidemiologic and immunologic features as well as reports on the microscopic appearance and biological

properties of the lymphoma tissue and cells. In contrast, detailed postmortem findings on large series of BL cases are very few in the literature.

The real extent of a widely disseminated disease which BL typifies can best be appreciated through postmortem examination of individual cases, particularly those who died before cancer therapy could be instituted. Such cases would be those presenting with advanced, fulminating, or widespread disease. Autopsy series are presumed to be biased for such cases. Despite such limitations, autopsy studies provide the most accurate assessment of relative predilection of tissues and organs for tumor formation or infiltration. Meaningful comparisons among series studied by the same or different workers can then be made of tumor distribution in the various abdominal, thoracic, and intracranial organs.

The pioneers of autopsy studies of BL were O'Connor (1961), Edington *et al.* (1964), and Wright (1964) who gave detailed accounts of postmortem findings on cases in Africa. Accounts of similar studies from the same or other high-incidence areas of the lymphoma are essential for comparison with the early reports and to further establish the pattern and spectrum of visceral organ involvement of the disease.

In this section, the postmortem observations of previous workers will be reviewed and compared with a similar study carried out in Ibadan, Nigeria (Osunkoya, 1973). In addition, other features of the general pathology of the lymphoma will be highlighted against the background of contemporary knowledge and thought on the disease.

2.2.1. Autopsy Studies

The series consists of (1) a retrospective analysis of the autopsy records of 45 cases of BL in the Department of Pathology, University College Hospital, Ibadan, from 1962 to 1964 and (2) a prospective study in the same department of

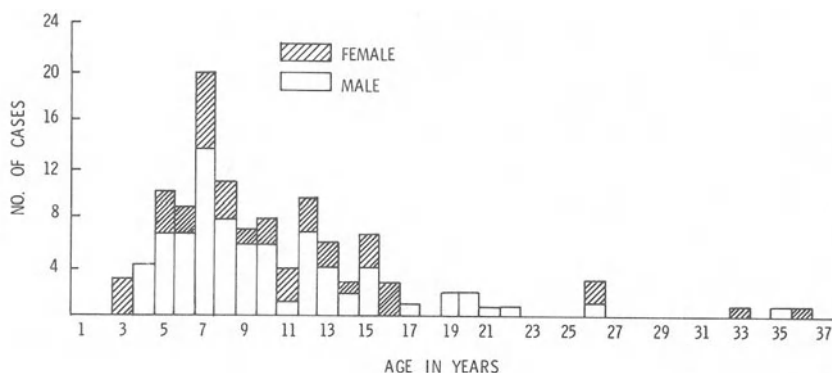


Figure 1. Age and sex distribution of 118 autopsy cases of Burkitt's lymphoma in Ibadan (1962-1970).

73 cases, most of which were studied by the author at postmortem examination from 1965 to 1970, inclusive. In all, there were 118 autopsy cases of histologically confirmed BL, 105 (88.9%) of which were children aged 16 years and below, with a distinct peak incidence at 7 years and a mean average age of 8.9 years. There were 80 male and 38 female cases with a sex ratio of approximately 2:1. The age and sex distribution of the cases are depicted in Figure 1.

The frequency of evident macroscopic tumor involvement of tissues and organs interest is as shown in Table I. Involvement of paired organs is analyzed in Table II.

Table I
Major Organ Distribution in 118 Autopsy Cases of
Burkitt's Lymphoma, Ibadan 1962-1970

Site of macroscopic tumor	No. of cases	Percent
Retroperitoneal tissues and related organs		
Retroperitoneal mass	61	51.0
Kidney(s)	77	65.2
Adrenal(s)	45	38.1
Pancreas	40	33.9
Liver	54	45.8
Spleen	44	37.3
Abdominal lymph nodes	57	48.3
Stomach	26	22.0
Intestines	37	31.4
Ovary ^a	19	50.0
Uterus ^a	5	13.2
Bladder	11	9.3
Spinal canal	6	5.1
Heart	37	31.4
Mediastinal lymph nodes	35	29.7
Facial bones and related organs		
Jaw(s)	24	20.3
Orbit(s)	15	12.7
Brain	17	14.4
Cervical lymph nodes	25	21.2
Other sites		
Thyroid	17	14.4
Other bones	24	20.3
Axillary lymph nodes	21	17.8
Inguinal lymph nodes	1	0.8
Breast(s) ^a	3	7.8
Testis(es) ^b	8	10.0
Lung(s) and pleura	2	1.7
Thymus	1	0.8

^aOf 38 female cases.

^bOf 80 male cases

Table II
Macroscopic Tumor Involvement of Paired Organs in 118 Autopsy Cases of
Burkitt's Lymphoma in Ibadan

No. of cases with tumor in one or both organs		Bilateral involvement		Unilateral involvement	
		No.	Percent	Right (no.)	Left (no.)
Kidneys	77	59	76.6	14	4
Adrenals	45	25	55.5	7	13
Ovaries	19	16	84.2	2	1
Testes	8	3	37.5	3	2
Orbits	15	3	20.0	6	6
Upper jaw	20	8	40.0	6	6
Lower jaw	11	5	45.6	3	3
Lungs	2	0	—	1	1

2.2.1a. Abdominal and Thoracic Tumors. Involvement of multiple abdominal organs occurs frequently and is indeed the most common feature of all series. The kidneys were the most frequently involved organs, usually in association with a retroperitoneal mass. About two-thirds (65.2%) of cases in the present series had macroscopic tumor in the kidneys. About half of the cases had tumor involvement of retroperitoneal tissues, abdominal lymph nodes, and the ovaries in the female group. At least one-third of the cases had tumor in the liver, spleen, adrenals, and pancreas. Other sites were less frequently involved.

Involvement of the kidneys, adrenals, pancreas, liver, spleen, stomach, and abdominal lymph nodes frequently occurred together, in association with a retroperitoneal tumor mass, the precise origin of which was not discernible. No abdominal organ is exempt from tumor involvement, and like the heart, involvement of the gastrointestinal tract, urinary bladder, and uterus is usually from the serosal tissues of the organ inwards. The whole thickness of the wall of such organs may, however, show tumor infiltration.

Tumor involvement of the kidneys, liver, and spleen is usually in the form of discrete nodules. The left lobe of the liver and the hilar portion of the spleen are the usual sites in these organs for such involvement. Occasionally in the liver, but not in the spleen, there is general massive enlargement with diffuse tumor tissue accentuating the portal tracts.

The only thoracic organs frequently involved by the tumor are the heart and mediastinal lymph nodes. In the heart, the tumor occurs as subepicardial nodules, especially in the atrio-ventricular groove, and also in the interventricular groove with varying degrees of extension of tumor tissue to involve underlying myocardium. Cardiac tumor involvement is frequently but not invariably associ-

ated with pericardial effusion. The thymus (Osunkoya, 1971), pleura, lungs, and thoracic cage are only rarely affected by macroscopic tumor.

The presence of a retroperitoneal mass (61 cases) was associated with macroscopic tumor involvement of one or more abdominal organs except in three cases in the frequency shown in Table III. Incidentally, all of the latter three cases had detectable tumor only as a retroperitoneal mass and tumor enlargement of mediastinal lymph nodes. Similarly, the frequent presence of tumor nodules in the heart is almost invariably associated with abdominal tumor. In only one out of the 37 cases with tumor involvement of the heart (Table I) was there no record of tumor in one or more abdominal organs or tissues, especially the retroperitoneal tissues and lymph nodes.

The serous cavities were frequently involved (Table IV) with mild to moderate effusions and, in some cases, small plaques of tumor tissue on the parietal wall of the cavities. Death resulted from acute peritonitis in two cases with tumor involvement of the stomach and perforated gastric ulcers.

In addition to the frequency of organ involvement listed in Table I, there was tumor involvement of the gall bladder in seven cases, all of which had tumor in the liver and other areas. The appendix was slightly enlarged by apparent diffuse tumor involvement in three cases, two of which were recorded not to

Table III
Tumor Involvement of Visceral Organs in Association with Retroperitoneal Lymph Nodes and Tumor Mass in 118 Autopsy Cases of Burkitt's Lymphoma, Ibadan, 1962-1970

	Retroperitoneal lymph nodes (no.)	Retroperitoneal tumor mass with or without lymph nodes (no.)	Either group	
			No.	Percent
Total cases	16	61	77	100
Female cases	7	16	23	30
Cases with tumor in:				
Kidney(s)	9	43	52	68
Adrenal(s)	7	32	39	51
Pancreas	6	29	35	45
Liver	7	30	37	48
Spleen	9	23	32	42
Stomach	4	15	19	25
Intestines	7	19	26	34
Ovary(ies)	5	8	13	57
Mediastinal lymph nodes	5	25	30	39
Heart	7	21	28	36

Table IV
Frequency of Serous Effusions in 118 Autopsy Cases of
Burkitt's Lymphoma in Ibadan

	No. of cases	Percent
Peritoneal effusion (ascites)	52 ^a	44.1
Pericardial effusion	45	38.1
Pleural effusion	40	33.9

^aOne case had chylous ascites.

have tumor in the intestines, although it was present in some other abdominal organs. The scalp was involved in three cases, all of which had tumor infiltration of the CNS.

2.2.1b. Gastrointestinal Tract. The stomach as an entity was the most frequent portion of the gastrointestinal tract (GIT) affected by tumor, usually in association with tumor in retroperitoneal tissues, pancreas, liver, and spleen. Of the 26 cases with tumor involvement of the stomach, as many as 15 had gastric ulcers. There was no apparent predilection of the lymphoma for any part of the small and large intestines. In particular, the terminal ileum and appendix were only rarely affected by tumor. As mentioned in a previous report (Osunkoya and Ajayi, 1972), tumor involvement of both small and large intestines is usually as round or oval plaques unrelated to Peyer's patches and more prominent on the serosal than the mucosal surface of the gut.

The relative frequency of involvement of the different parts of the GIT in the present series is depicted in Table V. Forty-three (36%) cases had tumor involvement of some portion of the GIT, and about half of these had ascites. The

Table V
Tumor Involvement of the Gastrointestinal Tract in Autopsy
Cases of Burkitt's Lymphoma in Ibadan

Site	Total cases with tumor ^a	Tumor at local GIT site only	Tumor at other GIT site also
Stomach	26	11	15
Small intestine	26	7	19
Appendix	3	2	1
Large intestine	23	4	19

^aNo. of cases with tumor at one or more GIT site is 43; no. of cases with GIT tumor associated with ascites is 21.

four cases with tumor affecting only the colonic part of the GIT were all females, three of whom had ovarian tumor at autopsy; it was not possible to determine whether or not the fourth case had tumor involvement of the ovaries before treatment.

2.2.1c. *Facial Tumors.* The detailed postmortem study and account made by Wright (1964) of the frequency of tumor involvement of the jaws and other facial bones of Ugandan cases give a clear picture of the postmortem appearances of the lymphoma in support of clinical and radiological features outlined in Section 2.1.4.

There was tumor involvement of the jaws in 24 (20.3%) cases. Table VI analyzes the frequency of jaw quadrant involvement in individual cases. In general, the maxilla was the most frequently involved facial bone. Direct spread of tumor beyond the jaw and other facial bones to involve orbital tissues, cranial nerves and intracranial tissues was evident in some cases in the manner described in previous autopsy series.

2.2.1d. *Central Nervous System.* Postmortem accounts of cases with involvement of the central nervous system (CNS) made by Wright (1964) and by Janota (1966) highlighted the frequency with which the tumor extends into the cranial cavity and spinal canal. The cases studied by Janota (1966) constitute part of the present series. Intracranial and intrathecal tumors were present in most cases in association with facial or retroperitoneal tumors, respectively, with varying degrees of tumor infiltration of cranial and spinal ganglia, nerve roots, meninges, brain, and pituitary.

Table VI
Involvement of Jaw Quadrants in 118
Autopsy Cases of Burkitt's Lymphoma in
Ibadan

Site	No. of cases
Maxilla	
Unilateral (1 quadrant)	9
Bilateral (2 quadrants)	4
Mandible	
Unilateral (1 quadrant)	3
Bilateral (2 quadrants)	3
Maxilla and mandible	
Unilateral	3
Bilateral (3 quadrants)	0
Bilateral (4 quadrants)	2
Total cases with jaw tumor	24

2.2.2. Histopathology

The histological appearance of Burkitt's lymphoma has been well described (O'Connor and Davies, 1960; O'Connor, 1961; Osunkoya, 1967a; Berard *et al.*, 1969; Wright, 1970) and is one of the well-known features of the disease. Tumor biopsy and autopsy materials show a remarkably constant histological feature of closely packed lymphoblasts interspersed by conspicuous large pale-staining histiocytes. The "starry-sky" effect which is thereby produced is characteristic but not pathognomic of BL and is reminiscent of the histological appearance of the germinal center of reactive lymph node follicles or malpighian corpuscles of the spleen. Starry-sky appearance is now accepted as typifying a group of lymphomas recently classified as centroblastic or follicular center cell lymphomas (Lukes and Collins, 1975).

The lymphoblasts of BL are closely packed cells with numerous although inconspicuous mitotic figures, indicating the highly malignant nature of the lymphoma. The histiocytic cells have a close resemblance to the dendritic histiocytes of lymphoid germinal centers. Like such cells, they regularly contain pyknotic debris of nuclei and even occasional intact lymphoblasts. Other cell types are not usually conspicuous, and like other lymphomas, blood vessels are not prominent. The tumor blast cells stain positively and uniformly with pyronin. Careful scrutiny of the blast cells reveals differences from the lymphoid cells of other types of centroblastic lymphomas. Stem cell lymphomas with starry-sky appearance as well as centrocytic lymphomas can be differentiated from BL.

Essentially, routine histopathological diagnosis of BL does not require more than differentiating the typical appearance of the tumor from other "round cell sarcomas" of childhood such as retinoblastoma, neuroblastoma, and nephroblastoma. Its distinction from Hodgkin's lymphoma, which has been observed (Edington *et al.*, 1973) to occur relatively frequently in children of school age, is also easy. However, cancer chemotherapy does affect the histological appearance of BL in such a way that the starry-sky effect may persist, but marked variation in the size of the lymphoid cells becomes evident. This may lead to diagnostic difficulties on autopsy material or biopsies taken during or shortly after a course of cytotoxic chemotherapy (Osunkoya *et al.*, 1970).

2.2.3. Cytology

Another aspect of the morphology of BL that has been studied and described in detail is the appearance of the tumor cells in cytological preparations. Two approaches have been used for the studies: imprint smear cytology popularized by Wright (1963) and phase contrast cytology used by Pulvertaft (1964) and Osunkoya (1970).

Good detail of the tumor cell morphology and some idea of the topography

of the tumor are obtained if a piece of biopsy tissue is pressed lightly on glass slides or cover slips. When dry, the imprint (impression or touch) smears thus prepared are fixed and stained like hematological preparations and may be mounted for microscopy and filing. The tumor cells appear as monomorphic lymphoblasts with distinct cytoplasmic vacuoles and intense cytoplasmic basophilia or pyroninophilia. A few small lymphocytes are present, and an occasional histiocytic cell is seen, usually containing pyknotic debris of cells.

Equally good cytological detail is obtained by phase contrast microscopy of slide preparations of the tumor cells in isotonic fluid medium or body fluid. Preparations may be made from tumor biopsies or needle aspirates of serous effusions, cerebrospinal fluid, or solid tumor. Light microscopy, especially by phase contrast, shows the tumor cells as round, noncoherent lymphoblasts with varying numbers of lymphocytes and histiocytic cells. Preparations may show rosette formation (peripolysis) with loosely adherent tumor blast cells surrounding a central histiocyte.

2.2.4. Electron Microscopy

Ultrastructural studies on BL biopsies have been undertaken extensively by several groups of workers primarily in search of virus particles. The features of the tumor lymphoblasts are typical of primitive lymphoid cells, with numerous free ribosomes in the cytoplasm. Mitochondria are scanty in most cells, and endoplasmic reticulum is poorly developed if at all. Virus particles have been observed by one group of workers within the nucleus and cytoplasm of cells from biopsy materials (Griffin *et al.*, 1966). Bedoya *et al.* (1969) highlighted the close approximation of the tumor lymphoblasts to histiocytes present in the tumor and noted that occasional tumor lymphoblasts with intact cell membranes were present within the cytoplasm of the histiocytes.

2.2.5. Biology of the Tumor Cells

A new era in tissue culture of human lymphoid cells was established by the simultaneous and independent reports by Pulvertaft (1964) and Epstein and Barr (1964) of continuous culture of lymphoblastoid cells from tumor biopsies. Apart from biopsies, ascitic and cerebrospinal fluids of BL patients have been used as the source of cell lines (Osunkoya, 1967c). Hundreds of lymphoblastoid cell lines have been established since then from BL patients and those with various other types of diseases as well as from healthy individuals by many laboratories. The cell lines have provided readily available material for a wide variety of biomedical research studies mentioned in Section 3.1.

The cell lines grow readily as suspension cultures in a wide variety of tissue culture media as nonadherent lymphoblastoid cells bearing a close resemblance

to mitogen-transformed lymphoblasts (Epstein and Barr, 1965; Osunkoya, 1967).

The most outstanding outcome of early studies on the cell lines from BL was the observation by Epstein (1964) of particles of a new species of herpes virus in the cultured cells by electron microscopy. The Epstein-Barr virus (EBV) was soon found to be a constant feature of human lymphoblastoid cell lines irrespective of the health status or type of disease of the individuals from which such cell lines are established. The EBV has been generally accepted to be the causative agent of infectious mononucleosis which, unlike BL, is a disease predominantly of young adults and relatively common in temperate climate. The role of the virus in the pathogenesis of BL is still unclear, although circumstantial evidence of its causative role is increasing, as reviewed a few years ago (De The, 1977).

The cells that constitute the tumor (lymphoblasts, lymphocytes, and histiocytes) have been largely characterized by immunologic parameters. The tumor lymphoblasts have the cell surface markers of B lymphoid cells (Jondal and Klein, 1973). Lymphocytes which are constantly present in the tumor were suspected to be of a different character from the tumor lymphoblasts (Osunkoya, 1967c). Although the tumor histiocytes do contain cellular debris, they were observed not to manifest phagocytic activity *in vitro* (Bedoya *et al.*, 1969). Short-term cultures of the tumor cells as well as early cultures that subsequently become established as lymphoblastoid suspension cultures regularly show monolayers of adherent "fibroblastic" spindle cells, some of which in all probability derive from the histiocytic cells of the tumor. The lymphoblastoid cells that constitute the continuous cell lines possess readily detectable cell surface markers characteristic of B lymphoid cells on the one hand and histiocytes on the other, suggesting that there is some degree of heterogeneity of closely related cell types in the population of cells in each cell line.

Early studies by several groups of workers showed that BL biopsy cells and lymphoblastoid cell lines release immunoglobulins into the medium in which the cells are incubated. The phenomenon has been generally interpreted as capacity for immunoglobulin synthesis and secretion by the lymphoblastoid cells of the tumor and additional evidence of the B cell origin of the tumor lymphoblasts (Osunkoya *et al.*, 1968).

Cytogenetic analysis of cells derived from the tumor led to the identification of chromosomal abnormalities which were found so regularly as to be proposed as markers for EBV-infected cells (Jacobs *et al.*, 1963; Miles and O'Neill, 1967; Gripenberg *et al.*, 1969; Fialkov *et al.*, 1972; Huber and Littlefield, 1975). Of particular significance in this respect is the abnormality affecting chromosome 14 of lymphoblastoid cells of EBV-positive cell lines from BL and infectious mononucleosis patients (Huang *et al.*, 1970). Furthermore, there is biochemical evidence for the presence of EBV genome in tumor tissue from BL patients (Hanson *et al.*, 1970; Dambaugh *et al.*, 1979).

2.2.6. Clinical Immunology

In a study of serum immunoglobulin levels of Nigerian BL patients, Ngu *et al.* (1966) observed significantly low IgM values in the patients as compared with healthy controls and other children with miscellaneous diseases. The finding has since been confirmed by groups of workers in other African countries (Ziegler *et al.*, 1970b; Nkrumah *et al.*, 1979). Serum levels of IgG and IgA do not show the same degree of difference between the patients and normal controls, whereas IgE and IgD values are yet to be reported on large groups of patients.

Specific antibodies to some microorganisms of interest have recently been estimated in the sera of BL patients in some detail. These relate to antibodies against viruses such as EBV, herpes simplex virus, and measles virus (De-Thé *et al.*, 1978), and malaria parasites (Nkrumah *et al.*, 1979). Although EBV antibodies are distinctly higher in BL patients than in age-matched controls from the same locality (De-Thé *et al.*, 1978), the patients have been reported to have significantly low material antibody titers (Nkrumah *et al.*, 1979).

Subtle changes have been noted in the parameters of T lymphocyte function in BL patients. Pretreatment T and B lymphocyte counts have shown trends of subnormal values for the patients and probably explain the same trend observed in phytohemagglutinin transformation tests carried out on patients (Cancedda *et al.*, 1974).

2.2.7. Clinical Biochemistry and Hematology

Pretreatment and serial blood urea and serum uric acid estimations are useful for proper clinical management of BL cases (Ngu, 1967). The urea level is a good monitor of renal function which is frequently abnormal because of extensive tumor infiltration of the kidneys or toxic side effects of drugs used for treatment of the disease. Slight to moderate elevation of uric acid levels is the rule, and marked elevation in suspected cases with no obvious tumor is highly suggestive of BL. Serum protein level and electrophoresis are generally normal.

Routine hematological values (hemoglobin levels, hematocrit, and leucocyte counts) in untreated cases are usually comparable to those of normal individuals of the same age group unless diffuse bone marrow infiltration by the lymphoma is extensive. The sickling test and hemoglobin electrophoresis may be crucial for diagnostic purposes to exclude hemoglobinopathy. In any case, examination of bone marrow aspirates is a valuable diagnostic investigation in all cases.

2.3. Clinicopathological Correlations

When considered together, the clinical and pathological features of BL highlight aspects of the disease that need to be clarified even as knowledge about

other aspects of the disease progresses. The unequivocal neoplastic nature of the disease will be discussed in this section as some of the clinical peculiarities of the disease as outlined above are correlated with pathological features.

2.3.1. Disease Entity

Since its first description by workers in Africa, the clinicopathological features of BL have been recognized to be in sharp contrast to those of leukemias and other lymphomas. The peculiar features are exemplified by the infrequency with which the disease primarily affects the peripheral blood or lymphoid organs. However, morphological and immunologic studies have clearly shown that the tumor consists of lymphoid cells and is therefore of hematopoietic origin. The rapidly proliferating lymphoid cells of the tumor attest to the highly malignant clinical course the neoplasm manifests.

An outstanding feature of BL is that it shows a constant histological appearance. Tumor tissue shows characteristic histological features of a follicular center cell (centroblastic) lymphoma irrespective of the mode of clinical presentation or site of the tumor provided that is no superimposed pathophysiological situation such as tumor necrosis because of ischemia or morphological cell changes from the effects of chemotherapy. Indeed, although not all centroblastic lymphomas are BL, the reverse is not true. Unlike BL, other centroblastic lymphomas present as classical lymphomas with neoplastic involvement of lymph nodes and spleen as the main feature. Burkitt's lymphoma should therefore be readily distinguishable on clinicopathological grounds from other lymphomas.

2.3.2. Multicentric Tumor

It is now generally appreciated that BL has diverse modes of clinical presentation. It is also well established that the lymphoma most often affects bones, particularly the jaws, and abdominal structures. A minority of cases will, however, continue to pose as differential diagnostic problems, especially if adequate tumor material is not procured or made available for diagnostic microscopy before treatment is instituted.

Radiological and postmortem anatomic observations have shown primary involvement of the marrow of affected bones and provide an explanation for the multiplicity of abdominal and thoracic organ involvement. The most plausible explanation offered to account for tumor spread from osteolytic primary sites in the jaw and other facial bones to involve orbital, intracranial, and other soft tissues of the head and upper neck is the direct spread of proliferating tumor cells through anatomic channels and tissue layers (Wright, 1964).

The involvement of multiple abdominal organs in individual cases can also

be explained simply as the result of direct spread of tumor cells through connective tissue planes, lymphatic channels, and in serous effusion of the peritoneal cavity. Such a manner of tumor spread is readily conceivable when, as is often the case, there is a "primary" retroperitoneal tumor mass. Centrifugal spread of tumor cells from such a site would involve not only abdominal viscera but also the epidural tissues of the spinal canal as well as midline structures in the posterior mediastinum. Indeed, the pericardial cavity, epicardial surface of the heart, and mediastinal lymph nodes are frequently involved by tumor in association with retroperitoneal and other abdominal structures. Tumor nodules or masses arising from each of the primary foci (bone marrow of facial and other bones and retroperitoneal mass) could manifest monoclonal features for each focus as indicated by the enzyme genetics studies of Fialkov *et al.* (1972). Satisfactory explanation is yet to be proposed for the development of BL in apparently aberrant sites such as the thyroid and breasts, beyond the possibility of tumor arising *de novo* at such sites.

2.3.3. Low Incidence of Associated Leukemia

Although BL is a type of "lymphosarcoma" frequently affecting bone marrow, the low leukemia tendency of the lymphoblastoid cells of the tumor is well known. The lymphoma may be regarded as a "malignant germinal center" (Osunkoya, 1967b), and, as mentioned earlier, current histological classification of the tumor is as a centroblastic lymphoma.

A prerequisite for the histophysiology and conceivably the pathophysiology of lymphoid germinal centers is close association of proliferating lymphoblasts with dendritic histiocytes. In other words, there is a functional requirement for close physical proximity and biochemical interrelationship between the lymphoblastoid cells and histiocytes of normal lymphoid germinal centers and (neoplastic) centroblastic lymphomas. Similarly, the pathophysiological unit of BL may be regarded as a central dendritic histiocyte with attached (peripoletic) lymphoblasts. Biochemical anchorage of the lymphoblastoid cells to fixed (tumor) histiocytes in the bone marrow and elsewhere would deter liberation of the blast cells into blood circulation with consequent leukemia. The nonleukemic tendency of multiple myeloma or Hodgkin's lymphoma bears a close analogy to this concept.

2.3.4. Renal Dysfunction

The elevated blood urea levels often seen in untreated BL patients are attributed to the very frequent involvement of both kidneys by the tumor. As pointed out by Biggar and Nkrumah (1979), this feature of the disease has therapeutic implications with regard to cytotoxic drug dosage. Renal dysfunction

induced by extensive tumor infiltration reduces capacity for drug clearance, with consequent manifestation of the effects of bone marrow depression by retained drugs.

2.3.5. Central Nervous System Involvement and Poor Prognosis

Infiltration of BL into the cranial cavity and spinal canal with cerebrospinal fluid leptomeningeal involvement and involvement of meninges, perivascular spaces, and neural tissues of the CNS is a well-recognized poor prognostic sign. Patients with such clinicopathological features have been categorized by all groups of workers as the worst cases in terms of response to chemotherapy, survival time, and clinical management. Many are cases that have relapsed after initial favorable response to therapy. Poor response to subsequent chemotherapy has been attributed in such cases either to selective persistence and continued proliferation of tumor cells with intrinsic low sensitivity to available drugs or to poor accessibility of drugs to tumor cells in the CNS imposed by the blood–brain barrier. Cytogenetic studies (Fialkov *et al.*, 1972) have indicated that cells with identifiable chromosome markers can indeed be selected by the internal environment of the patient (including presence of cytotoxic drugs) to persist and outgrow cell clones in the initial tumor.

2.4. Epidemiology

Soon after BL was described as a disease entity, Burkitt (1962a) undertook extensive incidence surveys of the disease in Uganda and other African countries. Similar epidemiologic studies carried out in other parts of the world established the uneven geographical distribution of the occurrence of the lymphoma as another striking feature of the disease. The early studies related the relative frequency of occurrence of the lymphoma to limiting geographical and climatic factors, and the observation prompted speculations and hypotheses on infective etiology of the disease. Much impetus for further epidemiologic and other types of studies have been generated by the hypotheses.

2.4.1. High- and Low-Incidence Areas

Typical BL cases have been noted in medical institutions in all continents as affecting children of human races. The lymphoma occurs relatively frequently in most tropical countries, especially in the tropical regions of Africa, South America, and in New Guinea. These areas have been described as “endemic” or high-incidence areas. Other areas where the lymphoma occurs in “sporadic” cases are referred to as low-incidence areas. It is pertinent to note that North America, where there is a large population of individuals of African descent, is geograph-

ically a low-incidence area of the lymphoma. It is also well established that the distribution of cases is quite uneven in the high-incidence areas of the disease (Booth *et al.*, 1961; Burkitt, 1961, 1962b,c,d; Burkitt and Wright, 1966; Kafuko and Burkitt, 1970).

Accurate national incidence rates of the lymphoma do not exist in most countries in the high-incidence areas because of lack of reliable vital statistics. The most practicable parameter that has been used until now for epidemiologic analysis is the frequency of the lymphoma relative to other types of cancer in the same locality. Relative frequency rates of the lymphoma were published about 20 years ago from cancer registries in Kampala, Ibadan, and Port Morsby, and similar figures became available from Monrovia recently. In all areas where the lymphoma occurs, it is clear that it is predominantly a disease of young people, especially children of school age.

Clustered occurrences of some BL cases have been reported from East Africa (Pike *et al.*, 1967; Morrow *et al.*, 1971). Such reports were concerned with the occurrence of the disease in siblings and close relatives with the implication of possible environmental and/or genetic factors in the causation of the disease.

2.4.2. Seroepidemiology

Several microorganisms have been associated with BL, with roles that are yet to be fully elucidated. Viruses and the *Plasmodium* of malaria are presently the most notable. Not long after the full clinical description of BL, Griffin *et al.* (1966) observed herpes-virus-like particles in tumor specimens from BL patients. It is also significant that since the first report of Epstein *et al.*, (1964), EBV has been a regular finding in cell lines derived from BL patients.

Of the putative infective agents associated with the etiology of the lymphoma, the foregoing reports contributed to the prominence of EBV as the agent on which attention was focused in seroepidemiologic studies. The most outstanding of such studies is the recently completed prospective population survey by De-Thé *et al.* (1978) in the Nile Valley districts of Uganda. The study indicates that BL patients have distinctly higher levels of EBV antibodies than do control groups of children long before their lymphoma becomes clinically detectable.

The indirect immunofluorescence test developed by Henle *et al.* (1969) was used initially by most workers for the detection and estimation of antibodies to EBV viral capsid antigen (VCA). Other tests specific for several EBV antigens have since been established. These include early antigens, membrane antigens (Henle *et al.*, 1971), complement-fixing soluble antigens (Sohier and De-Thé, 1972), and EBV-determined nuclear antigen (Reedman *et al.*, 1974). Serologic tests for all of these antigens were carried out in parallel on samples from individuals in the Nile Valley seroepidemiologic survey by De-Thé *et al.* (1978).

The results indicate that it is the relatively simple immunofluorescence test for VCA that most readily highlight the point that as a group, BL patients have higher EBV antibody titers relative to control children. Findings in Ibadan (B. O. Osunkoya and L. S. Salimonu, unpublished data) agree with the later observation. It is pertinent to note also that results of the above Nile Valley survey show little or no difference in the level and range of antibodies to measles virus, herpes simplex virus, and cytomegalovirus between BL patients and control children.

Evidence linking malaria with BL are mostly indirect and related to speculations based on geographical similarity in the occurrence of "endemic" malaria and BL (Kafuko and Burkitt, 1970; *Lancet*, 1970; Morrow *et al.*, 1976), as well as the possible effects of chronic (endemic) malaria on lymphoreticular tissues (Edington *et al.*, 1964; Dalldorf *et al.*, 1964; Edington, 1967). However, there is suggestion from a recent report from one group of workers that serum levels of IgM malaria antibodies specific for *P. falciparum* were lower in BL patients than in age- and sex-matched control neighborhood children (Nkrumah *et al.*, 1979). The longstanding observation of Jerusalem *et al.* (1971) of increased frequency of virus-induced lymphomas in mice with chronic malaria is in support of the association and remain to be explored further.

3. ADVANCES IN KNOWLEDGE OF THE DISEASE

World-wide interest in BL and the sustained impetus to further study of the disease in various institutions are provided by the known peculiarities of the disease. As outlined in the preceding sections of this chapter, such peculiarities have established BL as a malignant lymphoma commonly affecting very young people in relatively large numbers in tropical countries and showing epidemiologic features highly suggestive of infective etiology as well as good response to appropriate cytostatic chemotherapy. The peculiar pathological anatomic features of the lymphoma and successful continuous culture of lymphoid cells from the tumor made possible the discovery of EBV which has withstood the test of almost two decades of extensive studies as a highly putative causative agent of the disease.

Laboratory studies on BL in several institutions have opened up diverse areas of biomedical research, each with rapid accumulation of new findings related to immunology, genetics, and oncology in general. Such areas of research include diverse studies on lymphoblastoid cell lines, oncogenic properties of human herpes viruses, immunologic markers on the surface of lymphoid cells, and reclassification of malignant lymphomas. The latter became necessary when it was increasingly clear that BL does not strictly fall into any histological category of previously known lymphomas.

Although much work has been done on BL in many countries, the bitter

truth is that there have been no major breakthroughs in recent times in terms of knowledge of the etiology and pathogenesis of the disease. Broader concepts and new strategies are needed to further elucidate these aspects of the disease. The situation is indeed true of most human neoplastic diseases. However, new information on BL and related diseases is increasing steadily, and this makes possible the reappraisal of previous concepts about the disease as outlined in this section.

3.1. Characteristics of Burkitt's Lymphoma Tumor Cells

The immunologic markers of the lymphoid cells in BL have been well characterized by Klein and co-workers through studies carried out on biopsy cells (Klein *et al.*, 1977; Guven *et al.*, 1980; Galili *et al.*, 1980). The tumor cells were observed to have surface receptors for the γ -Fc piece and complement, and most specimens contained cells positive for surface IgM. κ - and λ -chain tests were reported to indicate monoclonal histogenesis of the lymphoma. In addition, the small lymphocytes present in the biopsies were observed to show characteristics of activated T lymphocytes.

A reappraisal of the interpretation of early studies on apparent immunoglobulin production by BL cells is warranted by the abovementioned recent findings. Detection of radioactively labeled immunoglobulins in the incubation medium containing cells labeled with radioactive amino acids could be a result of shedding into the medium of complexes comprising the radioactive labeled cell receptor together with adsorbed (unlabeled) immunoglobulins. This possibility is supported by the results of experiments carried out by B. O. Osunkoya (unpublished data) using BL cell lines. As mentioned in Section 2.2.5., the results of early studies were, however, interpreted as indicating synthesis and secretion of immunoglobulins by BL biopsy cells.

From the foregoing, it is obvious that the true nature and functional implication of the interaction among (1) dendritic histiocytes, (2) B-type lymphoblasts, and (3) T lymphocytes present in BL need to be fully elucidated. The nature of the interaction has a bearing on the etiology of the disease and the implication of the interaction could well be the basis of the pathogenesis of the lymphoma.

3.2. Hypotheses on Etiology of Burkitt's Lymphoma

Several attractive hypotheses have been put forward for the etiology of BL. Foremost among them are the insect-vectored oncogenic virus hypothesis of Harris (1964) and the malaria hypothesis of Dalldorf *et al.* (1964), Edington *et al.* (1964). In view of the present knowledge of the epidemiology of BL, what is also plausible is an infective agent that occurs both in tropical and temperate climates but is relatively common in the former. The putative agent should be vertically transmissible across the placenta and give rise to congenitally infected

infants. Apart from viruses, agents that meet these requirements are the protozoon of *Toxoplasma* as well as other closely related coccidia or leucocytozoon.

There are, indeed, three unpublished pieces of evidence in support of this possibility. First, *Toxoplasma* organisms have been detected in the monolayer ("fibroblast") portion of cells grown in tissue culture from biopsies of BL with the primary aim of establishing lymphoblastoid cell lines in suspension cultures (B. O. Osunkoya, unpublished observation, 1965). Second, and paradoxically, BL patients lack detectable antibodies to *Toxoplasma* by indirect immunofluorescence test in contrast to control children of the same age and from the same locality as the BL patients (B. O. Osunkoya, unpublished observation, 1971). Third, and rather tenuously, BL patients share with their respective mothers an unidentified soluble serum factor not present in the sera of their fathers. The soluble factor binds to the surface of cells from lymphoblastoid cell lines and leads to a cytotoxic reaction in the presence of complement. The factor was presumed to be antibodies (Osunkoya, 1966), but it could also well be soluble antigens binding to cell surface immunoglobulins now known to be present on the cells.

It is therefore still necessary to keep an open mind in terms of the actual infective agent(s) that are causally related to the initiation and pathogenesis of BL. Epstein-Barr virus remains a strong candidate.

3.3. Concepts of the Pathogenesis of Burkitt's Lymphoma

The technique of tissue typing to determine the histocompatibility antigen (HLA) profile of individuals has proved to be a useful tool for the assessment of genetic predisposition to various diseases including specific infections. It would appear that those infectious mononucleosis patients and Hodgkin's lymphoma patients showing high titers of antibody to EBV have outstandingly high concentrations of HLA-BW 35 specificity (for review see Jeannet, 1977). The genome allowing association (hybridization) with EBV on lymphoid and other cells is probably linked to that coding for HLA-BW 35 synthesis on the same cells in such a way that the antigenicity of EB viral antigens is enhanced. There is need for HLA typing of large numbers of BL patients and their relatives to confirm or refute the possibility that, as a group, the patients possess HLA-BW 35 or other HLA groups more so than appropriate control children. Such a study would be a follow-up of the analysis published by Bodmer *et al.* (1975).

The closer biochemical relationship of BL patients to their mothers than to their fathers, albeit poorly defined (Osunkoya, 1967), would suggest that genetic predisposition to BL is possibly a recessive X-linked trait. This possibility could also partly explain the higher frequency of the disease in boys.

It is not known precisely what mandatory factor for the pathogenesis of the disease, if any, is inherited by BL patients from their mothers. Is it a genetic

predisposition to EBV infection, or is it the virus itself that is vertically transmitted by BL patients from mother to fetus, or both?

Chronic virus infection of some cell types may predispose the infected cells to proliferation. Infectious mononucleosis is regarded to be the clinical manifestation of such an interaction between EBV and human lymphoid cells. The natural history of infectious mononucleosis as a self-limiting lymphoproliferative disorder could be the result of a prompt and effective immune reaction which eliminates the virus-infected cells.

Induction of neoplasia in cells chronically infected by a potentially oncogenic virus probably takes place during and as a result of a phase of immunosuppression in which the function and/or numbers of immune cells mediating surveillance are abruptly and drastically reduced (Jerusalem, 1968; Jerusalem *et al.*, 1971). Such an event could take the form of an acute infection by another agent with immunosuppressive properties. In the case of BL, such a tumor-initiating event could be acute infection of the "predisposed" child by malaria or, more likely, by other agent(s) endemic in high-incidence areas of the lymphoma. It is hereby suggested that such endemic agent(s) could be intracellular organisms with tropism for histiocytic cells.

Apparently normal small lymphocytes constantly present in EBV-associated tumors (BL and nasopharyngeal carcinoma) may well be playing the futile role of immune surveillance cells. The lymphocytes have been recently characterized as T cells by Klein and co-workers (Klein *et al.*, 1977; Galili *et al.*, 1980) and were noted by Osunkoya *et al.* (1970) to increase in numbers at the tumor sites of some BL patients during chemotherapy.

To fully appreciate the type of endemic infective agent(s) that would satisfy the criteria of the postulated cause of the tumor-initiating infection of the "predisposed" individual, it is necessary to consider the main sites of BL (jaws, orbit, and retroperitoneum). The agent(s) would need to have a predilection (tropism) for tissues of such sites. An agent that seems to meet these criteria is *Toxoplasma* or a closely related protozoon.

4. CONCLUDING REMARKS AND SUMMARY

In this brief review of well-recognized clinicopathological features of Burkitt's lymphoma (BL), emphasis has been placed on the description of findings in an autopsy series of 118 cases to highlight the existence of very few previous reports and the importance of such studies in contributing to better understanding of the disease. Discussion of probable etiologic factors and pathogenesis of BL in the light of advances in knowledge of the disease highlight the need for students of the disease to consider a wider variety of putative factors than has hitherto engaged the attention of workers. As with most neoplastic diseases, many ques-

tions about the cause(s) of BL remain unanswered, indicating the need for further studies.

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REFERENCES

- Ajayi, O. O., 1971, Gastrointestinal manifestations of Burkitt's tumour, *Ghana Med. J.* 10:30-33.
- Al-Attar, A., Al-Mondiry, H., Al-Bahrani, Z., and Al-Saleem, T., 1979, Burkitt's lymphoma in Iraq: Clinical and pathological study of 47 patients, *Int. J. Cancer* 23:14-17.
- Bannerman, R. H. O., 1966, Burkitt's tumour in pregnancy, *Br. Med. J.* 2:1136-1137.
- Bedoya, V., Grimley, R. M., and Rabson, A. S., 1969, Ultrastructural evidence of *in vitro* interaction among Burkitt's lymphoma cells: Possible relevance of the phagocytic activity of starry-sky histiocytes *in vivo*, *Cancer Res.* 29:753-762.
- Berard, C., O'Connor, G. T., Thomas, L. B., and Torloni, H., 1969, Histopathological definitions of Burkitt's tumour, *WHO Bull.* 40:601-607.
- Biggar, R. J., and Nkrumah, F. K., 1979, Tumour site and renal dysfunction as factors influencing leucopenia after chemotherapy for Burkitt's lymphoma, *Br. J. Cancer* 40:152-155.
- Biggar, R. J., Nkrumah, F. K., and Perkins, I. V., 1979, Presenting clinical features of Burkitt's lymphoma in Ghana, West Africa, *Trop. Pediatr. Environ. Child. Health* Dec., 157-161.
- Bluming, A. Z., and Ziegler, J. L., 1971, Regression of Burkitt's lymphoma in association with measles infection, *Lancet*, 2:105-106.
- Bodmer, J. G., Bodmer, W. F., Pickbourne, P., Degos, L., Dausset, J., and Dick, H. M., 1975, Combined analysis of three studies of patients with Burkitt's lymphoma, *Tissue Antigens* 5:63.
- Booth, K., Burkitt, D. P., Bassett, D. J., Cooke, R. A., and Biddulph, J., 1961, Burkitt's lymphoma in Papua, New Guinea, *Br. J. Cancer* 21:657-664.
- Brew, D., St., J., and Jackson, J. G., 1960, Lymphosarcoma in the ovary in young African girls in Nigeria, *Br. J. Cancer* 14:621-626.
- Burkitt, D. P., 1958, A sarcoma involving the jaws in African children, *Br. J. Surg.* 46:218-223.
- Burkitt, D. P., 1961, Observations on the geography of malignant lymphoma, *East Afr. Med. J.* 38:511-514.
- Burkitt, D. P., 1962a, A tumour syndrome affecting children in tropical Africa, *Postgrad. Med. J.* 38:71-79.
- Burkitt, D. P., 1962b, A children's cancer dependent on climatic factors, *Nature* 194:232-234.
- Burkitt, D. P., 1962c, Determining the climatic limitations of a children's cancer common in Africa, *Br. Med. J.* 2:1019-1023.
- Burkitt, D. P., 1962d, A tumour safari in East and Central Africa, *Br. J. Cancer* 16:379-386.
- Burkitt, D. P., and Wright, D. H., 1966, Geographical and tribal distributions of the African lymphoma in Uganda, *Br. Med. J.* 1:569-573.
- Cancedda, F., Osunkoya, B. O., Ngu, V. A., and Luzzatto, L., 1974, The response of peripheral blood lymphocytes of Burkitt's lymphoma patients to phytohaemagglutinin stimulation, *Niger. Med. J.* 4:72-75.

- Clifford, P., 1967, Observations on the treatment of Burkitt's lymphoma, in: *Treatment of Burkitt's tumour*, (J. H. Burchenal and D. P. Burkitt, eds.), Springer Verlag, Berlin, pp. 77-93.
- Cockshott, W. P., 1970, Radiological features of Burkitt's lymphoma, in: *Burkitt's Lymphoma*, (D. P. Burkitt and D. H. Wright, eds.), Churchill Livingstone, Edinburgh, pp. 23-33.
- Dalldorf, G., Linsell, C. A., Barnhart, F. E., and Martyn, R., 1964, An epidemiological approach to the lymphomas of African children and Burkitt's sarcoma of the jaws, *Perspect. Biol. Med.* 7:435-449.
- Dambaugh, T., Nkrumah, F. K., Biggar, R. J., and Kieff, E., 1979, Epstein-Barr virus RNA in Burkitt tumour tissue, *Cell* 16:313-322.
- De-Thé, G., 1977, Is Burkitt's lymphoma related to perinatal infection by EBV? *Lancet* 1:335-337.
- De-Thé, G., Geser, A., Day, N. E., Tukei, P. M., Williams, E. H., Beri, D. P., Smith, P. G., Dean, A. G., Bronkamm, G. W., Feorino, P., and Henle, W., 1978, Epidemiological evidence for causal relationship between Epstein-Barr virus and Burkitt's lymphoma from Ugandan prospective study, *Nature* 274:756-761.
- Durodola, J. I., 1976, Burkitt's lymphoma presenting during lactation, *Int. J. Gynaecol. Obstet.* 14:225-231.
- Edington, G. M., 1967, Pathology of malaria in West Africa, *Br. Med. J.* 1:715-718.
- Edington, G. M., Maclean, C. M. U., and Okubadejo, O. A., 1964, Necropsies on tumours of the reticuloendothelial system in Ibadan, Nigeria, with special reference to childhood lymphoma, in: *The Lympho-Reticular Tumours in Africa* (F. C. Roulet, ed.), Karger, Basel, p. 236.
- Edington G. M., Osunkoya, B. O., and Hendrickse, M., 1973, Histologic classification of Hodgkin's disease in the Western state of Nigeria, *J. Natl. Cancer Inst.* 50:1633-1637.
- Epstein, M. A., and Barr, Y. M., 1964, Cultivation *in vitro* of human lymphoblasts from Burkitt's malignant lymphoma, *Lancet* 1:252-253.
- Epstein, M. A., and Barr, Y. M., 1965, Characteristics and mode of growth of a tissue culture strain (EBI) of human lymphoblasts from Burkitt's lymphoma, *J. Natl. Cancer Inst.* 34:231-240.
- Epstein, M. A., Achong, B. G., and Barr, Y. M., 1964, Virus particles in cultured lymphoblasts from Burkitt's lymphoma, *Lancet* 1:702-703.
- Fialkov, P. J., Klein, G., and Clifford, P., 1972, Second malignant clone underlying a Burkitt tumour excretion, *Lancet*, 2:629-631.
- Gallil, U., Klein, E., Klein, G., and Bal, I. S., 1980, Activated thymus-derived lymphocytes in infiltrates and draining lymph nodes of nasopharyngeal carcinoma, *Int. J. Cancer* 25:85-90.
- Griffin, E. R., Wright, D. H., Bell, T. M., and Ross, M. G. R., 1966, Demonstration of virus particles in biopsy material from cases of Burkitt's tumour, *Eur. J. Cancer* 2:353-358.
- Gripenberg, U., Levan, A., and Clifford, P., 1969, Chromosomes in Burkitt's lymphoma: Serial studies in a case with bilateral tumours showing different chromosomal stem lines, *Int. J. Cancer* 4:334-349.
- Güven, P., Klein, G., Klein, E., Torstan, N., and Singh, S., 1980, Surface immuno-globulins on Burkitt's lymphoma biopsy cells from 91 patients, *Int. J. Cancer* 25:711-720.
- Hansen, H. zur, Schulte-Holthausen, H., Klein, G., Henle, W., Henle, G., Clifford, P., and Santesson, L., 1970, EB viral DNA in biopsies of Burkitt's tumours and anaplastic carcinomas of the nasopharynx, *Nature* 228:1056-1058.
- Henle, W., Henle, G., Clifford, P., Diehl, V., Kafuka, G. W., Kirya, B. G., Klein, G., Morrow, R. H., Munube, G. M. R., Pike, P., and Ziegler, J. L., 1969, Antibodies to Epstein Barr virus in Burkitt's lymphoma and central groups, *J. Natl. Cancer Inst.* 43:1147-1157.
- Henle, G., Henle, W., Klein, G., Güven, P., Clifford, P., Morrow, R. H., and Ziegler, J. L., 1971, Antibodies to early Epstein Barr virus-induced antigens in Burkitt's lymphoma, *J. Natl. Cancer Inst.* 46:861-871.
- Huang, C. C., Minowa, J., Smith, R. T., and Osunkoya, B. O., 1970, Re-evaluation of the relationship between chromosome marker and Epstein Barr virus: Chromosome and immu-

- nofluorescence analysis of 16 human haematopoietic cell lines, *J. Natl. Cancer Int.* 45:815–829.
- Hubuer, K. F., and Littlefield, L. G., 1975, Burkitt's lymphoma in three American children: Clinical and cytogenetic studies, *Am. J. Dis. Child.* 129:1219–1233.
- Jacobs, P. A., Tough, I. M., and Wright, D. H., 1963, Cytogenetic studies in Burkitt's lymphoma, *Lancet* 2:1144–1146.
- Janota, I., 1966, Involvement of the nervous system in malignant lymphoma in Nigeria, *Br. J. Cancer* 20:47–61.
- Jeannot, M., 1977, The main histocompatibility system in man, in: *Transplantation* (J. W. Masshoff, ed.), Springer Verlag, Berlin, Heidelberg, New York, pp.1–38.
- Jerusalem, C., 1968, Relationship between malaria infection (*P. berghei*) and malignant lymphoma in mice. *Trop. Med. Parasitol.* 19:94–108.
- Jerusalem, C., Jap, P., and Eling, W., 1971, Virus-induced malignant lymphoma in mice dependent on a RES conditioned by chronic parasitic infection (*P. berghei*), in: *RES and Immune Phenomena* (N. R. Diluzio, ed.), Plenum Press, pp. 391–399.
- Jondal, M., and Klein, G., 1973, Surface markers of human B and T lymphocytes. Presence of Epstein Barr virus receptors on B lymphocytes, *J. Exp. Med.* 138: 1365–1378.
- Kafuko, G. W., and Burkitt, D. P., 1970, Burkitt's lymphoma and malaria, *Int. J. Cancer* 6:1–9.
- Kaschka-Dierich, C., Adams, A., Lindahl, R., Bornkamm, G., Bjursell, G., Klein, G., Giovenella, B., and Singh, S., 1976, Intracellular forms of Epstein Barr virus DNA in human tumour cells *in vivo*, *Nature* 260:302–306.
- Klein, E., Svedmyr, E., Jondal, M., and Vanky, F., 1977, Functional studies on tumour-infiltrating lymphocytes in man, *Israel J. Med. Sci.* 13:747–752.
- Lancet*, 1970, Burkitt's lymphoma and malaria, *Lancet* 2:300–301.
- Lindahl, T., Klein, G., Reedman, B. M., Johansson, B., and Singh, S., 1974, Relationship between Epstein Barr virus (EBV) DNA and the EBV determined nuclear antigen (EBNA) in Burkitt's lymphoma biopsies and other lymphoreticular malignancies, *Int. J. Cancer* 13:764–772.
- Lukes, R. J., and Collins, R. D., 1975, New approaches to the classification of the lymphomata, *Br. J. Cancer* 31:1–28.
- Miles, C. P., and O'Neil, F., 1967, Chromosome studies of 8 *in vitro* lines of Burkitt's lymphoma, *Cancer Res.* 27:392–402.
- Morrow, R. H., Pike, M. C., Smith P. G., Ziegler, J. L., and Kisuule, S., 1971, Burkitt's lymphoma: A time-space cluster of cases in Bwamba country of Uganda, *Br. Med. J.* 2:491–492.
- Morrow, R. H., Kisuule, A., Pike, M. C., and Smith, P. G., 1976, Burkitt's lymphoma in the Mengo district of Uganda. Epidemiological features and their relationship to malaria, *J. Natl. Cancer Inst.* 46:479–483.
- Nug, V. A., 1967, The African lymphoma or the Burkitt tumour, *West Afr. Med. J.* 17:189–197.
- Ngu, V. A., 1968, The treatment of Burkitt's tumour, *West Afr. Med. J.* 17:273–280.
- Ngu, V. A., McFarlane, H., Osunkoya, B. O., and Udeozo, I. O. K., 1966, Immunoglobulins in Burkitt's lymphoma, *Lancet* 2:414–416.
- Nkrumah, F. K., and Perkins, I. V., 1973, Neurological manifestations of Burkitt's lymphoma in Ghana, *Afr. J. Med. Sci.* 4:209–214.
- Nkrumah, F. K., and Perkins, I. V., 1976, Burkitt's lymphoma. A clinical study of 110 patients, *Cancer* 37:671–676.
- Nkrumah, F. K., Sulzer, A. J., and Maddison, S. E., 1979, Serum immunoglobulin levels and malaria antibodies in Burkitt's lymphoma, *Trans. R. Soc. Trop. Med. Hyg.* 73:91–95.
- O'Connor, G. T., 1961, Malignant lymphoma in African children. A pathological entity, *Cancer* 14:270–283.

- O'Connor, G. T., and Davies, J., 1960, Malignant tumours in African children with special reference to malignant lymphoma, *J. Pediatr.* 56:526–535.
- Odeku, E. L., and Osuntokun, B. O., 1968, The clinical neurology of Burkitt's neoplasms. A preliminary study based on 105 cases, *West Afr. Med. J.* 27:263–268.
- Osunkoya, B. O., 1967a, The histopathology, cytology and immunology of Burkitt's lymphoma, *West Afr. Med. J.* 17:268–270.
- Osunkoya, B. O., 1967b, *Studies on the Burkitt and lymphoreticular tumours in Nigeria* Ph.D. Thesis, University of Ibadan, Ibadan, Nigeria.
- Osunkoya, B. O., 1967c, Observations on the establishment of Burkitt's tumour lymphoblasts in serially propagated cultures, *Cancer Res.* 27:2500–2504.
- Osunkoya, B. O., 1970, Short-term tissue culture, in: *Burkitt's Lymphoma* (D. P. Burkitt and D. H. Wright eds.), Churchill Livingstone, Edinburgh, pp. 134–147.
- Osunkoya, B. O., 1971, The thymus in Burkitt's lymphoma, *Niger. Med. J.* 1:154–157.
- Osunkoya, B. O., 1973, Burkitt's lymphoma: Autopsy and laboratory observations in Ibadan, Nigeria, in: *Proceedings 9th International Congress on Tropical Medicine and Malaria* (G. J. Papaevangelou, ed.) Athens, p. 94.
- Osunkoya, B. O., and Ajayi, O. O., 1972, Burkitt's lymphoma: A clinicopathological review of Ibadan cases, *Paediatrician* 1:261–272.
- Osunkoya, B. O., Mottram, F. O., Williams, A. I. O., and Ngu, V. A., 1968, Immunoglobulin synthesis by fresh biopsy cells and established cell lines from Burkitt's lymphoma, *Immunology* 14:851–859.
- Osunkoya, B. O., Ngu, V. A., and Mottram, F. O., 1970, Morphology of Burkitt's lymphoma during regression induced by cyclophosphamide, *Cancer* 25:505–509.
- Pike, M. C., Williams, E. H., and Wright, B., 1967, Burkitt's tumour in the West Nile district of Uganda, *Br. Med. J.* 2:395–399.
- Pulvertaft, R. J. V., 1964, Cytology of Burkitt's tumour (African lymphoma), *Lancet*, 1:238–240.
- Reedman, B. M., Klein, G., Pope, J. H., Walters, M. K., Hilgers, J., Singh, S., and Johansson, B., 1974, Epstein Barr virus associated complement-fixing and nuclear antigens in Burkitt's lymphoma biopsies, *Int. J. Cancer* 13:755–763.
- Shawker, T. H., Dunnick, N. R., Head, G. L., and Magrath, I. T., 1979, Ultra-sound evaluation of American Burkitt's lymphomas, *J. Cancer* 7:279–283.
- Sohier, R., and De The, G., 1972, Evolution of complement-fixing antibody titres with the development of Burkitt's lymphoma, *Int. J. Cancer* 9:525–528.
- Wright, D. H., 1963, Cytology and histochemistry of the Burkitt's lymphoma, *Br. J. Cancer* 17:50–55.
- Wright, D. H., 1964, Burkitt's tumour: A post-mortem study of 50 cases, *Br. J. Surg.* 51:245–251.
- Wright, D. H., 1970, Histopathology of Burkitt's lymphoma, in: *Burkitt's Lymphoma* (D. P. Burkitt and D. H. Wright, Eds.), Churchill Livingstone, Edinburgh, p. 77.
- Wright, D. H., and Pike, P. A., 1969, Bone marrow involvement in Burkitt's tumour, *Br. J. Haematol.* 15:409–416.
- Ziegler, J. L., Bluming, A. Z., Morrow, R. H., Fass, L., and Carbone, P. P., 1970a, Central nervous system involvement in Burkitt's lymphoma, *Blood* 36:718–728.
- Ziegler, J. L., Cohen, M. H., Morrow, R. H., Kwalwazi, S. K., and Carbone, P. P., 1970b, Immunologic studies in Burkitt's lymphoma, *Cancer* 25:734–739.
- Ziegler, J. L., Bluming, A. Z., and Morrow, R. H., 1972, Relapse patients in Burkitt's lymphoma, *Cancer Res.* 32:1217–1272.

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