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PARASITOLOGY

VOLUME 16

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PARASITOLOGY

Edited by

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PREFACE

It is a tribute to the excellence of the editorship of *Advances in Parasitology* by Professor Ben Dawes that I felt a certain pride at being invited by Academic Press to carry on the series when he died. I had frequently reviewed volumes of the series and recorded my high opinion of the quality of the contributions which Professor Dawes presented. The invitation was therefore a challenge which could hardly be declined. It is a further tribute to Professor Dawes that, in accepting the invitation of Academic Press, I felt that I could not undertake the task single-handed and made the proviso that I should need some support. In seeking this I have been most fortunate in attracting the aid of Drs J. R. Baker and R. L. Muller, both with extensive editorial experience added to established basic scientific reputations. I trust that, as a team, we may continue the high standard set by Professor Dawes single-handed.

Such is the time-scale of solicitation, editing and publication that the content of the present volume bears fundamentally the mark of Professor Dawes. It is a mix of Protozoology and Helminthology, with the latter predominating. Doubtless this same mix will continue to contribute largely to future volumes, but there may be an advantage—so as to provide freer flow of thought and concept—in interpreting "Parasitology" more widely than simply as Protozoology and Helminthology (together with Entomology, a discipline introduced mainly because arthropods provide most of the vector mechanisms), and bringing into consideration some of the manifold other kinds of organisms which, beside protozoa and helminths, follow the parasitic way of life.

W. H. R. LUMSDEN
August 1978

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Taxonomy and Transmission of *Leishmania*

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I. Introduction

The review by Adler (1964), by directing attention to "problems most likely to engage research workers in the near future", undoubtedly influenced many research programmes in the late 1960s and early 1970s and, for several years after his death, the genius of Saul Adler continued to act as a stimulus for many studies on *Leishmania.*

Because leishmaniases continue to be major public health problems in many parts of Asia, Africa, Europe and America, and because they are undoubtedly spreading (Anonymous, 1971), several important reviews on the subject have appeared in recent years. Reference should be made, in particular, to works by Bray (1972, 1974), Garnham (1971a,b), Heyneman (1971), Lainson and Shaw (1971, 1972, 1973, 1974), Lysenko (1971), Manson-Bahr (1971), Marsden and Nonata (1975), Moškovskij and Dunhamina (1971), Moškovskij and Southgate (1971), Neronov and Gunin (1971), Petriščeva (1971), Saf'janova (1971), Zuckerman (1975) and Zuckerman and Lainson (1977).

Lainson and Shaw (1971) succinctly reviewed the evidence incriminating sandflies (Diptera: Psychodidae—Phlebotominae) as the normal insect hosts

for *Leishmania* spp. Lewis (1971, 1974) dealt with the biology of Phlebotominae, with special reference to their rôle as vectors of leishmaniases. A book by Forattini (1973) contains a wealth of information about leishmaniases in the New World and the biology of Phlebotominae, especially that of Neotropical species. However the mass of facts was not so critically assessed as it was by Lainson and Shaw (1971, 1973, 1974), whose work now needs some revision in the light of later discoveries. The species of sandflies listed as vectors of leishmaniases by Bray (1974) are, in general, those which have been found naturally infected with promastigotes causing leishmanial infections when inoculated into susceptible laboratory animals; slight amendments in nomenclature of sandflies are needed and a few species can now be added.

Several reports, some with extensive bibliographies, on long term epidemiological investigations have also been published. Reference should be made to works summarizing studies in USSR (Perfil'ev, 1966; English translation, 1968), Belize (Lainson and Strangways-Dixon, 1963, 1964; Strangways-Dixon and Lainson, 1966; Disney, 1968; Williams, 1970), France (Rioux and Golvan, 1969), Sudan (Hoogstraal and Heyneman, 1969) and Ethiopia (Ashford *et al.*, 1973a). Studies on leishmaniasis in Panama, spanning about 30 years, have not yet been brought together in a single monograph; it is to be hoped that the important work carried out by the Wellcome Parasitology Unit in Belém will, eventually, be summarized in book form.

Whereas the review by Adler (1964) provided guidelines for future research, the present review attempts to summarize achievements in the last decade or so. Little reference is made to subjects authoritatively examined by the authors already cited. Only two topics are dealt with: taxonomy and nomenclature of *Leishmania*; and Phlebotominae as insect hosts for trypanosomatid parasites. Greater emphasis is given to the results of investigations in the New World. This, perhaps, reflects personal interests but it has been in the Americas that field studies have made great advances since the publication of Adler (1964).

II. Taxonomy and Nomenclature of *Leishmania*

The species of *Leishmania* infective to man are morphologically identical even though they give rise to a number of distinctly different clinical syndromes. The traditional methods for recognizing species were based on the disease states in man and, when urging the need for new standards for separation of species, Kirk (1950) commented that clinical differences were the only characteristics which represented "hereditarily stable differences in the parasites concerned". No modern student of *Leishmania* would give wholehearted support to such a statement; most would support Moškovskij and Southgate (1971) in considering clinical criteria, alone, as unsatisfactory taxonomic tools. In presenting a provisional classification of human leishmaniases on clinico-epidemiological evidence, Moškovskij and Southgate (1971) completely refrained from referring to the parasites by either generic or specific names.

It has become increasingly clear that a classification system based on human disease patterns has been a hindrance to understanding the variety of clinical forms of leishmaniases and to appreciating the specific and infraspecific relationships that exist between the parasites themselves. However the traditional framework for classifying *Leishmania* does not, in fact, conflict with modern methods for defining species and subspecies.

A. CLINICAL MANIFESTATIONS

Adler (1964) discussed the difficulties inherent in accepting a clinically-based taxonomy. He pointed out that, in all parts of the world where it occurs, clinically active visceral leishmaniasis presents the same symptoms; but, in different foci of infection, visceral manifestations may be preceded, accompanied or succeeded by cutaneous symptoms. He referred to cases from eastern Africa (Sudan and Kenya) in which patients may develop skin lesions, similar to oriental sore, several months before the onset of visceral signs of infection. Manson-Bahr (1955) referred to a report of the same phenomenon in southern USSR. Adler (1964) also discussed the difficulties in attempting to use clinical criteria to define the organisms responsible for the various forms of cutaneous and mucocutaneous leishmaniases in the Americas.

Cahill (1964) described cases of leishmaniasis acquired in an area of kala-azar in Upper Nile Province of Sudan by six North Americans. Four of the patients, described as healthy, well-nourished individuals, developed cutaneous lesions only. The three who had been taking antimalarial drugs presented a single lesion. The fourth, who had not been taking antimalarials, developed 18 separate lesions. The other two patients, who were older, in poorer physical condition and had not been taking antimalarial drugs, developed classical features of visceral leishmaniasis (fever, weight loss, anaemia, hepatosplenomegaly) without prior cutaneous manifestations. Subsequent serological and immunological studies on the parasites isolated from these patients revealed that the men were infected with the same strain (Adler *et al.*, 1966). The clinical manifestations in the patients were not due to distinctive properties of the parasites but depended on host characteristics—age, physical condition, dietary and drug (use of antimalarials) habits.

The use of serological and immunological techniques (Bray and Lainson, 1966, 1967; Bray and Rahim, 1969; Bray and Bryceson, 1969; Bray *et al.*, 1973a) established that diffuse cutaneous leishmaniasis, leishmaniasis recidiva and post-kala-azar dermal leishmaniasis are not produced by distinctive characteristics of the parasites concerned but represent different host reactions to them. Bray and Lainson (1966, 1967) could not differentiate between parasites isolated from a case of diffuse cutaneous leishmaniasis from the State of Pará, Brazil and *Leishmania* isolated from a case of espundia in the State of Ceará, Brazil. Bray and Rahim (1969) were unable to detect differences between the organisms isolated from cases of oriental sore and leishmaniasis recidiva in Iraq. (They showed, however, that organisms causing leishmaniasis recidiva in Iraq and Iran were distinctive and that the parasites causing oriental sore in Iraq and Ethiopia can be separated by serological tests.) Bray

and Bryceson (1969) proved that the same strain* of *Leishmania* in Ethiopia can cause diffuse cutaneous leishmaniasis, tuberculoid leishmaniasis, oriental sore and visceral leishmaniasis. Bray *et al.* (1973a) showed that the organisms causing kala-azar in India are serologically identical with parasites of post-kala-azar dermal leishmaniasis isolated from human cases in the same country.

Garnham (1971a,b) and Zuckerman (1975) reviewed the evidence that diffuse cutaneous leishmaniasis arises from a failure of cell-mediated immune processes in the human host. Zuckerman (1975) examined the evidence that leishmaniasis recidiva is the result of the development of hypersensitivity by the human host.

Apart from the advances in understanding the immunopathology and serology of *Leishmania* in man, a clinically-based taxonomical system of separating species would be realistic only if man were the prime mammalian host. In most cases, man is an incidental and, more often, an accidental, host of the parasites. With the exception of visceral leishmaniasis in the Indian subcontinent (and, perhaps, kala-azar in parts of eastern Africa), man is not the prime host of the parasites and, in fact, plays an insignificant rôle in their propagation. Although the genus *Leishmania* is in a state of active diversification, man is but one of many mammalian hosts for the parasites and has, probably, played little part in evolutionary sequences. As Bray and Lainson (1967) stated: "Speciation has occurred in rodents, canines and sandflies."

B. SEROLOGICAL AND IMMUNOLOGICAL STUDIES

Zuckerman (1975) reviewed recent studies on the immunology of leishmaniases, giving particular attention to the fundamental changes in ideas about immunopathological processes that have occurred since the subject was considered by Adler (1964). Here, reference is made only to some of the studies which have a bearing on understanding the taxonomic relationships of *Leishmania* spp.

1. *The Adler test*

Adler (1964) reviewed the earlier serological methods which had been used, often with inconsistent or contradictory results, to define species of *Leishmania;* and he described a technique which he had found useful in differentiating between the organisms causing espundia, chiclero's ulcer, oriental sore and kala-azar. The test also revealed slight but consistent differences between the parasites causing the moist and dry forms of oriental sore.

The Adler test entails growing a known and unknown *Leishmania* on media

* Following the recommendations of a meeting on the characterization, nomenclature and maintenance of salivarian trypanosomes held in London, 27–30 September 1976, under the chairmanship of W. H. R. Lumsden, the word "strain" as used throughout this paper should be replaced by "stock" ("population derived by serial passage *in vivo* and/or *in vitro* from a primary isolation, without any implication of homogeneity or characterization"). [Eds]

containing immune rabbit serum. Adler *et al.* (1966) and Garnham (1971a) described the sequence of events when parasites are seeded in cultures incorporating homologous or heterologous serum. Wertheim *et al.* (1970) studied the early extracellular and intracellular changes leading to the formations described by Adler (1964).

By use of this test, Adler *et al.* (1966) demonstrated that parasites isolated in Sudan from clinically different forms of leishmaniasis and from non-human sources were serologically similar. Strains from two human cases of kala-azar and three human cases of cutaneous leishmaniasis (isolated from five of the six cases described by Cahill, 1964), isolates from two naturally infected sandflies and from five naturally infected mammals (*Rattus rattus, Arvicanthis niloticus, Acomys albigena, Genetta genetta, Felis serval*) were shown to belong to the same species of *Leishmania*. The three cases of cutaneous leishmaniasis were considered to be examples of primary leishmanioma or abortive kala-azar.

Saf'janova (1966) used a modified form of the Adler test to distinguish between strains of parasites in sandflies, of human and lizard origins. He found that one species of fly had been infected with promastigotes which were antigenically close to human strains but that another species of fly had been infected with parasites of reptilian origin.

In Israel, Gunders *et al.* (1968) used the Adler test to identify strains of *Leishmania* isolated from *Meriones* (? species) and *Psammomys obesus*. The parasites from these two sources were indistinguishable from *Leishmania* isolated from a typical case of human oriental sore in Israel.

The Adler test provided a means of recognizing the origins of promastigote infections in sandflies, of identifying non-human hosts in a focus of human leishmaniasis and of the demonstrating that the same strain of parasite can give rise to distinct clinical conditions in man. In general the results tended to support the traditional clinically based taxonomic system for identifying the *Leishmania* infective to man.

2. *Fluorescent antibody staining technique*

Bray and Lainson (1965) demonstrated that this technique is of no value in identifying strains and species of *Leishmania*.

3. *Ouchterlony double diffusion tests*

Using this technique, Bray and Lainson (1966) found antigenic differences between strains but obtained insufficient evidence to identify parasites with certainty. They showed that a strain of parasite from Belize was distinct from strains isolated in Panama and Costa Rica; the Belize strain, isolated from a forest rat in an area of chiclero's ulcer, shared only one antigen with an Israeli strain of oriental sore even though the two strains had previously been found (Adler and Gunders, 1964) to produce cross-immunity; strains isolated from cases of visceral leishmaniasis in Brazil, Sudan, Kenya and India had three or more antigens in common with all other strains tested, including those

from cases (or from the areas) of cutaneous and mucocutaneous leishmaniases.

Schneider and Hertig (1966) found that antigenically distinct groups of *Leishmania* exist in Panama. Each of two groups included isolates from man and sandflies; the various strains of the two groups had no distinctive geographical patterns; both Panamanian groups were distinguishable from Guatemalan and Belize strains from cases of chiclero's ulcer, and from an old strain isolated from a case of uta in Peru. Schneider and Hertig (1966) were unable to group three isolates from Panamanian sandflies. The identity of one of these strains was later established by electron microscopy (Wallace and Hertig, 1968). By further double diffusion tests, Schneider (1968) showed that the other two ungrouped strains were closely related to a *Leishmania* which Herrer *et al.* (1966) isolated from a porcupine, *Coendou rothschildi*. Schneider found that the parasites from the porcupine were not closely related to those causing human cutaneous leishmaniasis in Panama; Herrer (1971) subsequently defined the porcupine parasites as *L. hertigi*.

4. *Cross absorption/passive haemagglutination test*

This test, using sensitized tanned sheep erythrocytes, was described by Bray and Lainson (1967) and Bray (1969).

Bray and Lainson (1967) found that the test consistently revealed antigenic differences between several of the strains examined but they refrained from naming the different serotypes demonstrated. The test confirmed the complexity of the situation existing in Central America: a strain from Panama was distinct from parasites isolated from a Brazilian case of espundia, from two strains from Costa Rica, and from a strain from Belize; the two Costa Rican strains were distinctive one from the other. Parasites from Indian kala-azar were differentiated from those of visceral leishmaniasis from Kenya. No antigenic difference was found between post-kala-azar dermal leishmanoid of India and oriental sore from Israel, or between typical espundia from the State of Ceará, Brazil and a case of diffuse cutaneous leishmaniasis from the State of Pará, Brazil.

Reference has already been made to results obtained by Bray and Rahim (1969), Bray and Bryceson (1969) and Bray *et al.* (1973b).

5. *Serological tests and the taxonomy of* Leishmania

The serological/immunological techniques used by Bray and his colleagues were aimed at the detection of humoral antibodies. Later studies showed that the immunopathology of leishmaniases can be better understood in terms of cell-mediated rather than humoral immunological processes (Zuckerman, 1975). The search for humoral antibodies, however, revealed consistent differences between certain strains, showed strain similarities even when the clinical syndromes differed considerably, and provided a more rational basis for understanding the relationships between strains of *Leishmania*.

C. GROWTH CHARACTERISTICS

Schneider and Hertig (1966) mentioned that some Panamanian strains of *Leishmania* did not thrive well in culture and could best be described as "slow growers". Bray and Mumford (1967) reported that a strain from Guyana grew slowly when inoculated into the nose of golden hamsters, the resultant lesion containing few amastigotes. The strain would not grow well in NNN medium containing rabbit blood and could be maintained only in medium prepared with hamster or rat blood.

Further evidence of differences in growth characteristics of *Leishmania* were obtained from studies on strains isolated in forested areas of Brazil and in Panama. Lainson and Shaw (1969a) briefly reported that *Leishmania* isolated from man and small forest rodents in the State of Mato Grosso, Brazil, could be separated into slow-growing and fast-growing strains. Further details were given by Lainson and Shaw (1970) and were later discussed by Lainson and Shaw (1971).

In the Mato Grosso study area, Lainson and Shaw (1969a; 1970) encountered both cutaneous and mucocutaneous lesions in man. Smears prepared from some single sore cutaneous lesions were found to contain many amastigotes whereas other, similar, lesions contained scanty amastigotes. Lesions found on small forest mammals were, with one exception, on the tail; in the exceptional case, the lesion was on the ear. All tail lesions were similar in appearance. Leishmanial lesions were found in 21 of the 107 small mammals examined (94 rodents, 13 marsupials); 20 of the infected animals were rodents and only one of the marsupials was infected. The behaviour of seven strains (one from a marsupial and six from rodents) in culture and after inoculation into golden hamsters was similar to that of strains isolated earlier in the State of Pará, Brazil (Lainson and Shaw, 1968, 1969b). The parasites grew profusely in cultures and no difficulty was experienced when transferring the organisms to new cultures; in hamsters, the parasites rapidly produced large histiocytomata containing many amastigotes. Three strains (one from a rodent, two from humans with single leishmanial lesions) behaved differently. Growth in culture was poor, transfer to new cultures proved difficult and the parasites often died after one or two transfers. When inoculated into hamsters, parasites developed slowly, producing small lesions containing few amastigotes; the lesions were surrounded by inflamed reactionary tissue. In comparing strains isolated from man and small forest mammals in the States of Mato Grosso, Pará and Maranhão, Lainson and Shaw (1970) found that both fast- and slow-growing organisms were isolated from single sore cutaneous lesions but only slow-growing parasites were recovered from cases of mucocutaneous leishmaniasis. In both man and hamsters, the slow-growing parasites provoked strong tissue reactions which were quite different from the host responses to fast-growing parasites. Lainson and Shaw (1970, 1971) discussed these findings in relation to the taxonomy of *Leishmania* in forested areas of Brazil but refrained from making new nomenclatorial proposals.

In Panama, Johnson and Hertig (1970) studied the growth characteristics

of dermatotropic *Leishmania* in laboratory-reared sandflies. Their observations, considered later (see p. 19), suggested that the position which promastigotes, of different strains of parasites, occupy in the digestive tract of sandflies could serve as a reliable taxonomic character for identifying strains and species.

Combining their own observations with studies in Panama, Lainson and Shaw (1972) presented a new scheme for classifying New World *Leishmania* spp. Their scheme was based, in part, on the growth characteristics of the parasites in man, in experimentally infected golden hamsters, in sandflies and in cultures.

Caution should be exercised in using growth characteristics as a taxonomic tool. Parasites isolated in areas of chiclero's ulcer in Belize have been successfully transmitted to man or golden hamsters by the bites of experimentally infected sandflies. In one of the successful transmission experiments, Coelho and Falcão (1962) found that lesions appeared on the hamsters 147–158 days after exposure to infected flies. The strain had been isolated from man in Belize in 1958, and maintained in laboratories in London and Jerusalem before it was received, in culture, in Belo Horizonte in 1961. In contrast, Strangways-Dixon and Lainson (1962, 1966), using a strain recently isolated from a forest rodent, reported development of a lesion (containing detectable amastigotes) only 17 days after a human volunteer had been bitten by an infected fly. Williams (1966) used the same strain as Strangways-Dixon and Lainson (1962, 1966) but after maintenance in the laboratory, mainly by passage through golden hamsters, for 2–2½ years. What were at first considered to be persistent reactions to sandfly bites were shown, by the detection of amastigotes four weeks after exposure, to be incipient leishmanial lesions. Growth patterns, at least in the mammalian host, can change during laboratory maintenance of a strain.

Growth characteristics can vary with recently isolated strains of the same parasite. Biagi *et al.* (1965; re-published 1966 and 1967 with no textual change) reported the development of a lesion after a human volunteer had been bitten by a naturally infected sandfly and also the results of inoculating a golden hamster with parasites from this fly. The rates of development of the lesions were not recorded but judging from the illustrations provided and guided by the actual publication date of Biagi *et al.* (1965), lesions must have developed in 2–3 weeks. On the other hand, lesions took six weeks to develop in two students who had helped in the field work.

Infectivity tests by Coelho *et al.* (1967e) indicated that growth rates may change after strains have been maintained in the laboratory for some time. In these observations, hamsters were inoculated with promastigotes recovered from the gut of experimentally infected sandflies. Seven hamsters inoculated with a strain isolated from a case of espundia developed lesions in 35–80 days; in all but one hamster, the lesions appeared within 35–43 days. Three hamsters inoculated with a strain isolated from a case of diffuse cutaneous leishmaniasis in the State of Bahia, Brazil, developed lesions in 65–109 days. Two hamsters inoculated with a strain isolated from a case of chiclero's ulcer in Belize developed lesions in 69 and 190 days. These results are the opposite of what

would be expected for growth rates of parasites causing espundia (slow-growing) and chiclero's ulcer (fast-growing). If the strains had not been confused during laboratory maintenance, then the results of Coelho *et al.* (1967e) strongly indicate that growth rates may be too variable to be of consistent taxonomic value.

Definition of growth patterns should, perhaps, be restricted to recently isolated or cryopreserved material. Methods of study should also be standardized so that growth rates can be defined with greater precision. Zeledón and de Monge (1967) described a method for comparing rates of increase of promastigotes in culture. Adaptation of the methods of Stauber (1966) could be used to estimate multiplication rates of amastigotes in mammalian skin and the subsequent metastatic spread that characterizes certain strains in infected golden hamsters.

D. BIOCHEMICAL STUDIES

The use of biochemical techniques in solving taxonomic problems of *Leishmania* represents an invaluable advance in studies on leishmaniases. The few reports now available are preliminary assessments, but such studies hold great promise for the future.

1. *Buoyant density of deoxyribonucleic acid (DNA)*

The morphological characters by which an organism is recognized and defined are expressions of the information contained within DNA. Techniques which reveal the base composition of DNA can be considered as extensions of traditional taxonomic methods because they define an organism's genetic attributes. Two organisms with dissimilar DNA base compositions are unrelated. Two organisms with similar DNA base compositions may or may not be closely related; base composition of DNA could be the same but the organization of information may be different. Kinetoplastida possess nuclear and extranuclear (kinetoplastic) DNA, referred to as NDNA (or n-DNA) and KDNA (or k-DNA); the base composition of DNA in the two systems can vary independently.

Schildkraut *et al.* (1962) were the first to suggest that studies on the base composition of DNA would be useful in solving problems in the taxonomy of Protozoa. The value of studies in elucidating the relationships within Kinetoplastida have recently been examined by Newton (1976). Studies on the genus *Leishmania* were begun by Morales *et al.* (1972), Chance (1972) and Chance *et al.* (1973). A comparative study, involving the examination of more than 70 strains, was published by Chance *et al.* (1974).

In the strains studied by Chance *et al.* (1974), buoyant density of n-DNA varied from 1·714 to 1·720 g ml^{-1} and that of k-DNA between 1·691 and 1·707 g ml^{-1}. The data were tabulated on a lattice to indicate groupings of strains and species. Mammalian and lizard species of *Leishmania* were separable; *L. hertigi* was distinct from all other mammalian species; dermatotropic *Leishmania* from the Old and New Worlds were distinct; the fast- and

slow-growing American strains of Lainson and Shaw (1970) were distinct; distinctions were made between dermatotropic *Leishmania* from different parts of the Old World. Observations on organisms causing visceral leishmaniasis gave less conclusive results; some organisms, which can be separated on geographical grounds and for clinico-epidemiological reasons had similar DNA base compositions.

2. *Enzyme electrophoresis*

Gardener and Howells (1972) reported that disc polyacrylamide electrophoresis of malate dehydrogenase (MDH) could be a sensitive tool for distinguishing species and strains of *Leishmania* and they identified five different MDH variant patterns among the strains examined. In a more detailed report. Gardener *et al.* (1974) recognized 13 different categories amongst 73 strains studied.

The results of DNA analysis (Chance *et al.*, 1974) and MDH electrophoresis (Gardener *et al.*, 1974) supported each other in most cases; strains with distinctive DNA often have different MDH types. Some strains with identical DNA were separable by MDH electrophoresis. By combining the results of the two different biochemical methods, the traditional species were subdivided into two or more distinct groups but it was thought to be premature to decide if these groups represent distinct geographical races, subspecies or species.

Some additional means of enzyme characterization of *Leishmania* were mentioned by Gardener *et al.* (1974). Thin layer starch gel electrophoresis revealed species-characteristic variants of alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) in *Leishmania* (Kilgour *et al.*, 1974) and the results indicated a means of differentiating between four strains of fast-growing dermatotropic *Leishmania* from the Americas.

These studies on enzymes were made on promastigotes from cultures. Mattock (1975) tested amastigotes of two strains of *Leishmania*, grown in mouse peritoneal macrophages and dog sarcoma cells. Polyacrylamide gel disc electrophoresis failed to detect MDH or lactate dehydrogenase (LDH) in the parasites.

3. *Biochemistry and taxonomy*

Lumsden (1974) advocated that the definition of biochemical characteristics should form the background for examining all other information about *Leishmania* strains and urged that cloned and cryopreserved material should be used in future studies. Several of the strains studied by Chance *et al.* (1974) and Gardener *et al.* (1974) had been maintained in laboratories for many years; the Liverpool strain LV 23, for example, was isolated about 30 years previously. The histories of several strains were unknown or had been lost through frequent transfers from one laboratory to another. The Liverpool strain LV 22, the only strain in MDH group II, was originally isolated from a case of chiclero's ulcer in Belize and passed through five laboratories (in

sequence: London, Jerusalem, Belo Horizonte, Basle, Liverpool), crossing the Atlantic three times as it was distributed from place to place, and had been variously maintained in each laboratory. Clearer results were obtained with recently isolated or cryopreserved strains.

Biochemical studies can reveal the true identity of strains isolated from unusual sites in unusual hosts; the Liverpool strains LV 12 and LV 61 provide an example. Both strains were isolated from the kidney of two foxes (*Cerdocyon thous*) from the State of Pará, Brazil, and both were found, by biochemical methods, to be related to fast-growing dermatotropic strains from rodents and man in the same area. Gardener *et al.* (1974) suggested that the strains might have been confused in the laboratory of origin, but this seems unlikely. It is more likely that Lainson *et al.* (1969) discovered a dermatotropic *Leishmania* which became a visceral parasite in an unusual host. If this is so, the biochemical studies cast doubts on the suggestion by Lainson *et al.* (1969) and Lainson and Shaw (1971, 1972, 1973, 1974) that a reservoir host for visceral leishmaniasis exists in forested areas of South America. Isolates from human visceral infections in Brazil show no relationship to cutaneous strains from the New World but share characteristics with Old World strains, especially with organisms of visceral leishmaniasis from East Africa and India. These results reopen questions about the origin of visceral leishmaniasis in the Americas.

When it is becoming possible to define strains and species of *Leishmania* by very sensitive biochemical methods, confusion may arise from the designations of isolates. In tabulating details of strains, Chance *et al.* (1974) referred to three different codes by which some strains have been designated. The problems have been discussed by Killick-Kendrick (1976) and Lumsden (1976); a workable and unified system for strain designation is needed.

E. MORPHOLOGY

Apart from the amastigotes of *L. enriettii* and *L. hertigi* (neither of which is infective to man) it is often stated that all other *Leishmania* parasitic in mammals are identical when viewed under the light microscope. Shaw and Lainson (1976) established that consistent differences exist between amastigotes of fast- and slow-growing dermatotropic *Leishmania* from northern Brazil. The fast-growing strains have an amastigote of mean size 3·44 × 2·2 μm, with the kinetoplast more central and the nucleus more posterior. The slow-growing strains have a smaller amastigote (2·4 × 1·75 μm) and the nucleus and kinetoplast are closer together.

Using a carefully calibrated electron microscope, Chance and Gardener (1975) examined sections of amastigotes which contained a nucleus and were cut transversely to the sub-pellicular microtubules. Four sizes of amastigote were recognized, the groupings of strains closely corresponding to those determined by DNA buoyant density measurements and MDH electrophoresis variants.

In general, the results of electron microscope studies, reviewed by Adler

(1964), Garnham (1971a) and Bray (1974), have not yet revealed well-defined ultrastructural differences between *Leishmania* species, though Sanyal and Sen Gupta (1967) and Gardener (1974) suggested that specific differences in the numbers and spacing of sub-pellicular microtubules may exist. With these possible exceptions, the schematic diagram of the fine structure of an amastigote given by Bray (1974) is representative of all species of *Leishmania* infective to man.

F. CLASSIFICATION OF THE GENUS *LEISHMANIA*

Mammals and reptiles are the only known vertebrate hosts for *Leishmania*. Some lizard *Leishmania* occur in areas of human leishmaniasis and, because the parasites develop in phlebotomine sandflies, it is sometimes necessary to determine if promastigotes in the gut of flies are of mammalian or reptilian origin. Some species of lizard *Leishmania* share antigens with species infective to mammals and can produce cryptic and transient infections in mammals (Adler, 1964; Belova, 1971).

1. *Parasites infective to mammals*

The most recent attempts to classify mammalian *Leishmania* in the traditional way (that is, by naming and defining species and subspecies) were presented by Lainson and Shaw (1972, 1973, 1974) and Bray *et al.* (1973a). Notes on the distribution, taxonomy, mammalian and sandfly hosts of each species and subspecies were given by Bray (1974). Vickerman (1976) recognized four species complexes within the genus: the *donovani* complex, for organisms causing human visceral leishmaniases; the *tropica* complex, for organisms causing a variety of cutaneous syndromes in man in the Old World; the *mexicana* complex, for the fast-growing organisms in the Americas which usually cause, in man, single sore lesions; the *braziliensis* complex, for the slow-growing organisms which, in the New World, cause single sore and mucocutaneous lesions in man.

In Table 1, species complexes are listed in the order given by Vickerman (1976) and, apart from the *braziliensis* complex, species within each complex are listed in the chronological order of original definition. Arranged in this way, it is clear that close concordance exists between the traditional and biochemical methods for identifying species and subspecies.

Clinico-epidemiological, serological and immunological evidence, as well as the results of biochemical studies, suggest further subdivision of all complexes (except, perhaps, the *braziliensis* complex); and there is little doubt that there are some species whose existence had not previously been suspected. A fast-growing strain from the State of Goiás, Brazil, differs in DNA values and MDH type from all other American isolates (Gardener *et al.*, 1974). Parasites isolated in the State of Minas Gerais, Brazil, have been found, by biochemical methods, to belong to the *mexicana* complex (W. Peters, personal communication). This greatly extends the geographical range of the complex to an area whose landscape (predominantly developed agricultural land) is very different

TABLE 1
Leishmania *infective to mammals*

Complex/species/subspecies	n-DNA[a] (g ml^{-1})	k-DNA[a] (g ml^{-1})	MDH type [b]
Donovani complex			
L. donovani (Laveran and Mesnil)	1·719	1·703–1·707	V, VII
L. infantum	1·719	1·703–1·704	V, XI
L. chagasi da Cunha and Chagas	1·719	1·702–1·704	VII
Tropica complex			
L. tropica (Wright)	1·719	1·703–1·707	I, IV
L. major Yakimov and Schochov	1·718	1·703	I
L. aethiopica Bray, Ashford and Bray	1·719	1·706	V
Mexicana complex			
L. enriettii Nuniz and Medina[c]	1·718	1·701	V
L. mexicana mexicana Biagi	1·718	1·700	I, II
L. mexicana amazonensis Lainson and Shaw	1·718	1·697–1·699	III
L. mexicana ssp. indet. (from Panama)	1·718	1·698	I
Braziliensis complex			
L. peruviana Velez	—	—	—
L. braziliensis panamensis Lainson and Shaw	1·717	1·694	0
L. braziliensis guyanensis Floch	1·716	1·692	X
L. braziliensis braziliensis Vianna	1·716	1·691	VI
Unplaced species			
L. hertigi Herrer[c]	1·714	1·700	IX

a Chance *et al.* (1974).
b Gardener *et al.* (1974).
c Not infective to man.

from the densely forested regions associated with other members of the *mexicana* complex. A strain isolated from the State of Bahia, Brazil, has been reported as belonging to the *mexicana* complex (Brazil, 1974) but, because the subspecies was not defined, the proposed name must be considered *nomen nudum* (White, 1976).

2. *Parasites infective to lizards*

Adler (1964) stressed that studies on lizard *Leishmania* might throw light on the evolution of the genus as a whole. The most intensive and extensive investigations on these parasites in recent times have been carried out in Turkmenian SSR, and a summary of this work was given by Belova (1971).

Table 2 lists, in the chronological order in which they were described, the 10 known species of lizard *Leishmania* but, as pointed out by Garnham (1971a), some names may be synonymous. Information about the original hosts from which the parasites were described and distribution is derived, except for the species described by McMillan (1965), from Adler (1964) and Garnham (1971a). The species from the Old World are known, or are thought likely, to develop in the gut of sandflies (see p. 25). The only New World

species was described from the island of Martinique, where only one species of sandfly has been recorded (Floch and Abonnenc, 1952).

Chance *et al.* (1974) and Gardener *et al.* (1974) studied seven isolates of lizard *Leishmania*. *L. agamae* and *L. tarentolae* had similar DNA values and were the only species in MDH group VIII. Three strains of *L. adleri* had buoyant density values of 1·716 g ml^{-1} (n-DNA) and 1·700 g ml^{-1} (k-DNA) and fell, with strains of *L. mexicana amazonensis*, into MDH group III. Another strain of *L. adleri* and one of *L. hoogstraali* had buoyant density values of 1·716—1·717 g ml^{-1} (n-DNA) and 1·703–1·704 g ml^{-1} (k-DNA) and, with strains of the *donovani* complex, belonged to MDH group VII.

TABLE 2

Leishmania *infective to lizards*

Species	Host	Distribution
L. henrici Leger	*Anolis* sp.	Martinique
L. chamaeleonis Wenyon	*Chamaeleon vulgaris*	Egypt, Israel
L. tarentolae Wenyon	*Tarentola mauritanica*	North Africa, Sicily
L. hemidactyli Mackie, Gupta and Swaminath	*Hemidactylus gleadovii*	India
L. agamae David	*Agama stellio*	Israel, Turkmenian SSR
L. ceramodactyli Adler and Theodor	*Ceramodactylus dorias*	Iraq, Turkmenian SSR
L. gymnodactyli Khodukin and Sofiev	*Gymnodactylus caspius*	Turkmenian SSR
L. adleri Heisch	*Latastia longicauda*	Kenya
L. zmeevi Andrushko and Markov	*Eremias intermedia*	Turkmenian SSR
L. hoogstraali McMillan	*Hemidactylus turcicus*	Sudan

L. adleri, as presently defined, is probably a composite species with affiliations with members of the *donovani* complex isolated within the same geographical area and, on the other hand, a strange relationship with a fast-growing dermatotropic mammalian *Leishmania* from north Brazil. The taxonomic link between *L. adleri* and the *donovani* complex was anticipated by observations reviewed by Adler (1964). The link between *L. adleri* and *L. mexicana amazonensis*, particularly because lizard *Leishmania* has not been found in continental America, deserves further study. It may or may not be significant that *L. adleri* has factors in common with the *donovani* complex in eastern Africa, and with *L. mexicana amazonensis* which, in an unusual host, can produce visceral infections in organs not usually invaded by parasites of the *mexicana* complex. The evidence that *L. chagasi* from Brazil is related to *L. donovani* from eastern Africa, that *L. adleri* is related to both east African *L. donovani* and *L. mexicana amazonensis* and that strains of *L. mexicana amazonensis* can cause visceral leishmaniasis in canines, could be a connecting link in understanding the origins of human visceral leishmaniasis in the Americas.

The biochemical relationship of *L. adleri* and *L. hoogstraali* with the *donovani* complex in eastern Africa, coupled with the evidence (Adler, 1964) that *L. adleri* can cause transient and cryptic infections in mammalian hosts (including man) and further coupled with the evidence that *L. adleri* is transmitted by a sandfly which feeds on reptiles and mammals, supports previous suggestions that these two lizard *Leishmania*, both occurring in areas of human visceral leishmaniasis, can confer immunity to kala-azar. In contrast, *L. agamae* and *L. tarentolae* are not closely related to mammalian *Leishmania*; if inoculated into man, they are unlikely to provoke antibody reactions to overwhelm later invasions by *Leishmania* of the *donovani* and *tropica* complexes.

III. Phlebotominae as Hosts for Trypanosomatidae

Adler (1964) and Lainson and Shaw (1971) reviewed early studies incriminating phlebotomine sandflies as normal insect hosts of *Leishmania*. The evidence is now overwhelming that sandflies are the prime vectors of all forms of leishmaniases. There is also an increasing volume of evidence that Phlebotominae are the natural insect hosts of several other genera of Trypanosomatidae.

A. Nomenclature and Biology of Phlebotominae

Adler (1964) referred to all sandflies by the generic name *Phlebotomus* and a few students of Phlebotominae still recognize this as the only genus within the subfamily. Most writers on sandflies, however, follow the suggestion of Theodor (1948) who separated the subfamily into four genera: *Phlebotomus* and *Sergentomyia* for Old World species; *Lutzomyia* and *Brumptomyia* for New World species. The three large genera (*Phlebotomus, Sergentomyia* and *Lutzomyia*) are usually subdivided into formally defined subgenera and a number of less formal species-groups.

Soon after the proposals of Theodor (1948) were made, *Warileya* and *Hertigia* were defined (Hertig, 1948; Fairchild, 1949) as generic names for some American sandflies. As a result of studies on Bolivian Phlebotominae (Velasco and Trapido, 1974), the generic status of *Hertigia* is doubtful, but it is here retained as a generic name.

Forattini (1971, 1973), in an attempt to simplify the classification of American sandflies, increased the number of genera and decreased the number of subgenera. Names applied by most authors to subgenera are often used as generic titles by those following Forattini's system of classification. Forattini's system is too cumbersome to be useful and tends to mask natural relationships between species. Treatment of American sandflies based on the proposals of Theodor (1965) is preferable.

The appearance of the mouth parts of the only known species of *Hertigia* suggests that the females have blood feeding habits but the preferred hosts have not been discovered. The genus is known from a very few specimens collected in Panama and Costa Rica. Four species of *Warileya* have been

described, specimens coming from high or very high altitudes in Costa Rica, Panama, Colombia, Peru and Bolivia. Females of two species readily attack man when given the opportunity to do so, but no organism pathogenic to man has been isolated from *Warileya*. The genus *Brumptomyia* is widely distributed, from Mexico to Argentina, through forested and wooded areas of the Neotropical Region. The distribution of *Brumptomyia*, therefore, closely coincides with the distribution of American cutaneous leishmaniases but there are no authentic records of female *Brumptomyia* biting man. In South America (but not in Central America), species of *Brumptomyia* can be collected in greatest numbers from armadillo burrows. There is strong circumstantial evidence that female *Brumptomyia* feed exclusively on armadillos but only Mangabeira (1942) actually witnessed them doing so.

Species of *Sergentomyia* occur in foci of leishmaniasis in the Old World but, in general, the genus occurs in greater numbers and with greater diversity in areas south of the main centres of leishmaniases. Female *Sergentomyia* usually feed on cold-blooded vertebrates but some species feed on mammals, including man. Some species of *Sergentomyia* may be involved in the transmission of mammal *Leishmania* but promastigotes found in the gut of wild-caught specimens are often derived from lizards.

Vectors of leishmaniases in the Old World belong to the genus *Phlebotomus*. Species of the subgenera *Phlebotomus, Paraphlebotomus, Synphlebotomus* and *Larroussius* have been incriminated as vectors of cutaneous leishmaniases; members of the subgenera *Synphlebotomus, Larroussius, Adlerius* and *Euphlebotumus* have been associated with kala-azar (Lewis, 1974).

All proved vectors of leishmaniases in the Americas are members of the genus *Lutzomyia*. The vector of American visceral leishmaniasis is a member of the subgenus *Lutzomyia*; many other species of this subgenus readily attack man when given the opportunity to do so, but only one species is undoubtedly associated with kala-azar in the New World. Most vectors of American cutaneous leishmaniases belong to the subgenera *Psychodopygus* or *Nyssomyia*. In southern Brazil, a species of the subgenus *Pintomyia* (as well as one of *Nyssomyia*) has been shown to be the insect host of *Leishmania*, probably of the *braziliensis* complex. None of these subgenera occur in zones of uta; the suspected vectors of this disease belong to the *verrucarum* group. A member of the *cruciatia* group, and a species which cannot be placed in a subgenus or species-group, have been found infected with promastigotes in Panama.

B. *LEISHMANIA* IN EXPERIMENTALLY INFECTED SANDFLIES

Experimental studies on the development of *Leishmania* in phlebotomines were reviewed by Adler and Theodor (1957). Briefly, the amastigotes develop into promastigotes which multiply in the stomach of the sandfly; many promastigotes migrate forwards and become attached, by the tip of the flagellum, to the epithelium of the cardiac stomach and to the proventricular valve; further forward migration takes the parasites into the pharynx, cibarium and, sometimes, to the distal tips of the mouth parts. Adler (1964) considered

this to be the normal growth pattern of promastigotes of mammalian *Leishmania*. Adler and Theodor (1957) also considered that mammalian *Leishmania* have a vector specificity and that different species of sandflies are not equally susceptible to infection.

Some recent experimental work supports the statements of Adler and Theodor (1957) and Adler (1964) but other studies have shown that some New World *Leishmania* have a different growth pattern in the gut of sandflies and that many species of *Lutzomyia* are equally susceptible to infection.

1. *Experiments with* Phlebotomus

In a focus of visceral leishmaniasis in Sudan, Hoogstraal *et al.* (1962) established that *P. orientalis* (= *P. langeroni orientalis* of many authors) was the main man-biting species in *Acacia* forest but that *P. papatasi*, rare in woodland, was a troublesome biter in villages. Other evidence suggested *P. orientalis* as the likely vector.

When females of *P. orientalis* were infected with a Sudan strain of *L. donovani* (McConnell, 1964), promastigotes were found concentrated in the cardiac stomach 6–7 days after the infecting blood meal; in some specimens, the parasites were packed in solid, immobile masses near the proventricular valve. When *P. papatasi* were infected, 10 000 or more promastigotes per fly were found in females dissected on the first two days after infection, 5000–10 000 on the 3rd–4th days and only 100 by the 5th day (Heyneman, 1963). The decline in intensity of infection was due to elimination of promastigotes through the hind gut; it was concluded that *P. papatasi* is a poor host for *L. donovani* in Sudan. Davis (1967) found that structural differences in the alimentary tracts of *P. papatasi* and *P. orientalis* could account for differences in the behaviour of promastigotes in the two species of flies.

In Ethiopia, field studies by Lemma *et al.* (1969) suggested that *P. longipes* is the likely vector of *L. aethiopica*. Foster (1972) fed female *P. longipes* on patients with various clinical manifestations of the parasite. Promastigotes were found in 27·2% of the fed flies. Highest concentrations of parasites were in the mid gut, most usually in the cardiac stomach. Promastigotes were seen in the hind gut only when they also occurred, in greater numbers, in the mid gut. Infections anterior to the proventricular valve were found, seven or more days after the infecting blood meal, in 37% of the infected flies. Some flies with anterior gut infections harboured promastigotes in the hind gut as well.

2. *Experiments with* Lutzomyia

The lack of host specificity of *Leishmania* to American sandflies was first demonstrated by the independent observations of Hertig and McConnell (1963) and Coelho (1964).

Hertig and McConnell (1963) worked with several strains of *Leishmania* isolated in Panama. All strains readily developed in *Lu. panamensis, Lu. trapidoi, Lu. ylephiletor, Lu. gomezi* and *Lu. sanguinaria*, the five commonest

man-biting sandflies in forests of Panama. Coelho (1964) carried out experiments with a strain of *Leishmania* isolated in Belize (= *L. mexicana mexicana*) and a strain isolated from a case of diffuse cutaneous leishmaniasis in the State of Bahia, Brazil. The second strain was referred to as *L. braziliensis* but has since been recognized (Brazil, 1974) as a member of the *mexicana* complex. Both strains of parasite readily developed in *Lu. longipalpis* and *Lu. renei*, sandflies which do not occur in the areas where the parasites were isolated.

The ease with which different *Leishmania* can develop in different species of *Lutzomyia* was further demonstrated by studies in Belize and additional observations in Brazil. Strangways-Dixon and Lainson (1962, 1966) reported that *L. mexicana mexicana* developed in nine species of sandflies captured on man in forest in Belize: *Lu. bispinosa, Lu. geniculata, Lu. pessoana* (probably an incorrect identification), *Lu. panamensis, Lu. olmeca, Lu. ylephiletor, Lu. cruciata, Lu. ovallesi* and *Lu. shannoni*. With the possible exception of *Lu. olmeca*, all species extend well beyond the known geographical limits of *L. mexicana mexicana* infections.

Coelho *et al.* (1967a) reported that a strain of the *mexicana* complex would develop in eight species of sandflies: *Lu. intermedia, Lu. whitmani, Lu. sallesi, Lu. shannoni, Lu. arthuri, Lu. monticola, Lu. misionensis* (called *Lu. coelhoi—nomen nudum*—in the original paper) and *Lu. cavernicola*. Coelho (1966) and Coelho *et al.* (1967b) found that Old World *Leishmania* can develop in *Lu. longipalpis* and *Lu. renei*; according to Bray (1974), the Zamira and Ein Geddi II strains used in these experiments were *L. tropica* and *L. major*, respectively. *Lu. longipalpis* and *Lu. renei* were also experimentally infected with *L. chagasi* (isolated in the State of Minas Gerais, Brazil) and with parasites from a case of mucocutaneous leishmaniasis (presumably of the *braziliensis* complex) from the State of Ceará, Brazil.

In further experiments undertaken in Panama, Johnson and Hertig (1970) showed that strains of *Leishmania* from Guatemala, Belize and Peru, as well as parasites isolated in Panama, readily develop in *Lu. gomezi* and *Lu. sanguinaria*.

In comparison with the conclusions which could be drawn from the studies of Heyneman (1963) and McConnell (1964) in Sudan, experimental infections in various species of *Lutzomyia* have failed to demonstrate differences in their capacities to act as hosts for *Leishmania*.

3. *Behaviour of* Leishmania *in the sand fly gut*

The growth patterns of Sudanese *L. donovani* in *P. orientalis* (McConnell, 1964) and of *L. aethiopica* in *P. longipes* (Foster, 1972), in general, conformed with those described by Adler and Theodor (1957). In five species of *Lutzomyia* from Panama, Hertig and McConnell (1963) observed multiplication of promastigotes in the stomach followed by anterior migration. They reported that multiplication in the stomach took place during the first three days of infection. On the 3rd day, but sometimes sooner, promastigotes reached the cardiac stomach, concentrating near or at the proventricular valve. Some-

times as early as the 3rd day, and frequently on the 4th and 5th days, the cardiac stomach was packed with promastigotes. Invasion of the fore gut was first seen on the 3rd day of infection, with more promastigotes migrating beyond the proventricular valve on the 4th and 5th days. Fore gut infections were established only when promastigotes, often in the form of solid masses, were also present near the proventricular valve. In flies with fore gut infections, the walls of the oesophagus were sometimes distended; the pharynx of some flies contained massive numbers of promastigotes.

The surprising feature of the experiments by Hertig and McConnell (1963) was the high frequency of promastigotes in the hind gut. Invasion of the hind gut began on the 3rd day, coinciding with the appearance of promastigotes in the cardiac stomach. The pyloric portion of the gut (the "hind triangle" of Hertig and McConnell, 1963 and Johnson and Hertig, 1970) was the region where the greatest numbers of promastigotes became attached to the gut epithelium, but attached promastigotes were seen throughout the intestine as far as the rectal ampulla. Unattached promastigotes were also found in the lumen of the hind gut. In several flies, the wall of the pylorus was covered with attached promastigotes and the lumen was packed with unattached forms. Once established, hind gut infections persisted throughout the life of the fly and did not indicate—in contrast to Sudan *L. donovani* in *P. papatasi* (Heyneman, 1963)—that the parasites were being eliminated.

In studies on the development of *L. mexicana mexicana* in sandflies caught in Belize, Strangways-Dixon and Lainson (1966) noted several similarities but some important differences from the results obtained by Hertig and McConnell (1963). Strangways-Dixon and Lainson (1966) described the development of *L. mexicana mexicana* as "explosive". Multiplication began when the parasites were still amastigotes; transformation to promastigotes occurred 24–36 hours after the parasites had been ingested and, thereafter, multiplication was profuse. By the 4th–5th days after infection, the mid gut and fore gut were packed with active flagellates and, in some flies, the proboscis had already been invaded. Promastigotes were never seen attached to the gut wall, nor did they form compacted masses in the gut lumen, but were actively free-moving. Strangways-Dixon and Lainson (1966) occasionally noted promastigotes in the lumen of the hind gut but never observed parasites attached to the hind gut epithelium.

When *Lu. longipalpis* and *Lu. renei* were infected with *L. tropica, L. mexicana* and *L. chagasi,* Coelho *et al.* (1967b,c,d) reported promastigotes in the hind gut of the flies. With a single exception, hind gut infections were noted in only 5–11% of infected flies and occurred only when promastigotes were in greater numbers in the mid gut. Hind gut infections of *L. chagasi* in *Lu. renei* might have indicated—as in the case of Sudan *L. donovani* in *P. papatasi* (Heyneman, 1963)—that the parasites were being eliminated; only a few dozen promastigotes were found in the flies, and none was infected on or after the 6th day following the infecting blood meal.

Johnson and Hertig (1970) examined the significance of hind gut infections in American Phlebotominae. Laboratory reared *Lu. gomezi* and *Lu. sanguinaria* were infected with strains of dermatotropic *Leishmania* from Peru,

Panama, Guatemala and Belize. Promastigotes were found in the hind gut of 8% of flies infected with the Guatemalan strain; similar results were obtained in the few trials carried out with the Belize strain. On the other hand, more than 90% of the flies infected with the Peruvian and Panamanian strains had promastigotes attached to the epithelium of the hind gut, with most parasites in the pylorus. Purely hind gut infections were found in about 50% of flies infected with strains from Peru and Panama but about half the flies had promastigotes in the mid gut (and sometimes in the fore gut) as well as in the hind gut. Although the Panamanian and Peruvian parasites multiplied in the hind gut, especially in the pylorus, they aggregated in the anterior part of the alimentary canal of the sandflies and, from there, could be inoculated into mammalian skin when next the fly attempted to suck blood.

Johnson and Hertig (1970) concluded that the mode of transmission of American *Leishmania* by sandflies does not differ from that of Old World species of the parasites but that different growth patterns of dermatotropic *Leishmania* in *Lutzomyia* spp. could provide an additional means of separating and defining strains from different parts of the New World. Lainson and Shaw (1972, 1973, 1974) followed this suggestion when they used growth patterns in sandflies as one of the criteria for separating the *mexicana* and *braziliensis* complexes.

In a report on electron microscope studies on the development of *L. mexicana amazonensis* in laboratory reared *Lu. longipalpis*, Killick-Kendrick *et al.* (1974) used the terms nectomonads and haptomonads to refer to different forms of promastigotes found in the gut of sandflies. Nectomonads are slender promastigotes which are attached to the gut epithelium of the insects; haptomonads are thicker and shorter promastigotes that become attached only to the cuticular lining of the oesophageal valve. Killick-Kendrick *et al.* (1974) observed that amastigotes transformed into long thin promastigotes which rapidly multiplied in the stomach, some becoming embedded in the peritrophic membrane or being attached to the membrane by the flagellum. When the remains of the blood meal passed into the hind gut, the nectomonads became attached to the gut wall by inserting the flagellum between the microvilli of the mid gut epithelial cells. Killick-Kendrick *et al.* (1975) reported that nectomonads, when viewed by a scanning electron microscope, have a body length of 11 μm with a flagellum 11 μm long, whereas haptomonads are 7 μm long with a long flagellum 11 μm in length. Molyneux *et al.* (1975) described ultrastructural differences between nectomonad and haptomonad promastigotes. These structural differences probably indicate differences in basic physiological processes in the two forms of promastigotes. They also reported that the forms attached to the cuticular wall of the pharynx were not typical promastigotes but resembled opisthomastigotes.

The application of electron microscope studies to the forms of *Leishmania* which occur in the digestive tracts of sandflies provides means of distinguishing between the different physiological processes that the parasites must pass through in the insect host before they become infective to mammalian hosts. Electron microscope studies on the parasites in an insect host might provide

evidence for morphological and behavioural differences at subspecies level. Using the light microscope, Strangways-Dixon and Lainson (1966) recorded that promastigotes of *L. mexicana mexicana*, in the gut of species of *Lutzomyia*, are unattached and free-living in the lumen of the gut. In contrast, the scanning electron microscope studies of Killick-Kendrick *et al.* (1975) showed that most promastigotes of *L. mexicana amazonensis*, in the stomach of *Lu. longipalpis*, were attached.

4. *Infectivity of promastigotes in sandflies*

Hertig and McConnell (1963) experienced difficulties in infecting mammals with promastigotes recovered from the gut of experimentally infected sandflies. One suckling white mouse was found infected three days after inoculation with promastigotes from an infected *Lu. sanguinaria*. This was their only successful transfer of parasites from insect to mammalian hosts. No infection was detected in other baby mice inoculated with promastigotes from the gut of experimentally infected *Lu. sanguinaria* and *Lu. gomezi*. No lesion appeared in two golden hamsters inoculated with the gut contents of 13 experimentally infected *Lu. gomezi*; promastigotes were in the cardiac stomach of all the infected flies and the pharynx was heavily infected in two of them. Promastigotes from experimentally infected *Lu. sanguinaria*, *Lu. gomezi*, *Lu. panamensis* and *Lu. trapidoi* failed to induce leishmanial infections in *Proechimys semispinosus*, a species of rat which had earlier been found (by culture of heart blood) to be naturally infected with *Leishmania* in Panama.

The difficulties experienced in Panama might reflect the methods used to infect sandflies. At the time of the experiments of Hertig and McConnell (1963), no laboratory animal had been found susceptible to Panamanian strains of dermatotropic *Leishmania*. Sandflies were infected by artificially feeding them, through a micropipette fitted closely around the proboscis, on promastigotes from cultures. Multiplication of promastigotes in the gut of the sandflies might well have been a continuation of the reproductive processes initiated in the cultures from which the parasites were derived. Promastigotes which develop in cultures are not necessarily the equivalents of promastigotes which pass through morphological and physiological sequences in the gut of a sandfly.

In contrast to the difficulties experienced in Panama, Coelho (1964) found that promastigotes of American dermatotropic *Leishmania* became infective to golden hamsters after only two days of development in the gut of sandflies. In later experiments, Coelho *et al.* (1967e) inoculated hamsters with the gut contents of *Lu. longipalpis* and *Lu. renei* experimentally infected with a strain of the *braziliensis* complex, with two different strains of the *mexicana* complex, with *L. tropica* or with *L. chagasi*. Eighteen out of 37 hamsters died without developing signs of infection; most deaths occurred within a few days of inoculation and could have been due to concomitant or secondary infections. Surviving hamsters were kept under observation for 18 months. Leishmanial infections developed in 16–43% of the animals originally inoculated and in 84% of those which survived for 18 months. In these

experiments, sandflies were not artificially infected but were allowed to take blood directly from the lesions of infected laboratory animals.

5. *Transmission of* Leishmania *by the bites of infected sandflies*

Difficulties have always been experienced in demonstrating the actual transfer of *Leishmania* by the bites of infected sandflies. Recent successful transmission experiments have all involved parasites of the *mexicana* complex. The sandflies which transmitted the parasites were *Lu. longipalpis, Lu. renei, Lu. pessoana* (more probably *Lu. panamensis), Lu. cruciata* and *Lu. olmeca* (called *P. flaviscutellatus* in the original report) (Coelho and Falcão, 1962; Strangways-Dixon and Lainson, 1962, 1966; Biagi *et al.*, 1965; Williams, 1966, 1970). In the experiments of Biagi *et al.* (1965), the sandfly was naturally infected; in the other experiments, the flies had been infected in the laboratory. In the experiments of Strangways-Dixon and Lainson (1962, 1966) and Williams (1966, 1970), transmission occurred less than four days after the flies had been infected and without an intervening blood meal. Transmission occurred when the flies only probed the skin, without ingesting blood.

Although inoculation of promastigotes by probing sandflies is the most likely way by which non-human mammals become infected with *Leishmania*, this is not necessarily true for human infections. The few observations that have been made on the bites of Phlebotominae, together with notes on the reactions to bites, strongly suggest that probing flies have little opportunity to deposit the parasites. Adler and Theodor (1957) referred to the needle-like pain induced by sandflies. Williams (1966) commented that bites of some *Lu. cruciata* were painful throughout the time the mouth parts were inserted in the skin. Hoogstraal and Heyneman (1969) likened the bites of *P. orientalis* to "fiery-sharp . . . needle pricks" and they noted that some collectors "complained quickly, loudly, and unhappily when a sandfly bit them". Some collectors "scratched afflicted areas . . . so vigorously that pale abrasions persisted on their dark skins for some minutes"; this reaction could "play some role in the spread of *Leishmania* organisms in the body immediately after inoculation by *P. orientalis*". The comment can be made that, had the men not been engaged in the collection of sandflies, they would probably have slapped the bitten area while the fly was probing, thus spattering the gut contents of the fly around the puncture wound. If the fly were infected, the subsequent scratching of the skin would be the actual means by which the parasites are introduced into human tissues.

Slapping and scratching the area of skin probed by a sandfly is an almost universal reaction; it is difficult to resist doing so even when collecting flies in areas where sandflies occur in large numbers! It seems likely that the majority of human leishmanial infections are the results of squashing probing flies rather than inoculation of parasites by the flies themselves.

6. *Development of* L. enriettii *in sand flies*

Adler (1956) reported that *L. enriettii* multiplied profusely in the Old World sandfly *P. papatasi*, but observations were made only during the five days after infection. Hertig and McConnell (1963) artificially fed *L. enriettii* to five *Lu. sanguinaria* and four *Lu. gomezi*. When dissected, no promastigote was found in any of the *Lu. sanguinaria* or in two *Lu. gomezi*. The other two *Lu. gomezi* were found to be heavily infected when they were dissected 11 and 12 days after exposure to the parasites. In one fly, promastigotes were attached near or at the proventricular valve; in the other, they were seen only in the stomach.

Coelho (1966) fed 326 *Lu. longipalpis* and 34 *Lu. renei* on laboratory guineapigs with *L. enriettii* lesions. Only 2·5% of *Lu. longipalpis* and 11·8% of *Lu. renei* became infected; the low infection rates were in sharp contrast to the very high infection rates when the two species of flies were infected with other *Leishmania* spp. Only a few promastigotes (1–2 dozen) were found in flies dissected 2–4 days after infection; the parasites were seen only in the stomach. Although some flies survived for 10–12 days, no infection was found after the 6th day; the disappearance of promastigotes occurred at the time that the peritrophic membrane was eliminated. In both species of flies, the promastigotes of *L. enriettii* were elongate, with a very short flagellum and moved extremely slowly.

Luz *et al.* (1966/67) reported that *L. enriettii* developed well in *Lu. monticola*. (Throughout the original paper, the specific name was given as "*monticula*" and it was again misspelt by Brazil (1975). From the evidence of geographical distribution (Martins *et al.*, in the press), the species used in the experiments was unlikely to have been *Lu. monticola* but was probably the undescribed female of *Lu. paulwilliamsi*.) Forty wild-caught females were fed on infected guineapigs. Ten flies were dissected 2–5 days after the blood meal and promastigotes were found in six. The parasites were said to occur in all parts of the digestive tract, including the proboscis. No detail was given about the relative abundance of parasites in different parts of the gut and no reference was made to their morphology and activity.

Because so little is known about the origin of *L. enriettii*, Luz *et al.* (1966/67) using sandflies collected near the laboratory where the parasite was discovered, could have provided vital information. Unfortunately, they made observations only on a small number of flies and their results were vaguely and confusingly presented. Further observations on *L. enriettii* in sandflies, using the methodology of Johnson and Hertig (1970), would be valuable.

C. NATURAL *LEISHMANIA* INFECTIONS IN SANDFLIES

Adler (1964) advised against triturating batches of sandflies and inoculating the resultant suspensions into susceptible laboratory animals. He argued that valuable information can be lost by using this method. Triturating groups of sandflies can, however, be of value in certain circumstances.

Use of this method enabled Strangways-Dixon and Lainson (1962) to pinpoint a forest focus of *L. mexicana mexicana* in Belize. The studies on rodents

in this area (Lainson and Strangways-Dixon, 1964) profoundly changed concepts on the epidemiology of American cutaneous leishmaniases. In Sudan, Hoogstraal and Dietlein (1963) and Hoogstraal and Heyneman (1969) found that triturating small batches of specifically identified flies, when supported by dissection of individual specimens, gave a reliable index of infection rates.

Dissection of individual sandflies is by far the best method to discover vectors of leishmaniases. Because, normally, very few infected sandflies are captured, many specimens need to be dissected before reliable infection rates can be calculated. The tedium of examining many negative flies can be overcome if, concurrently, observations are made on internal structural features of the flies with a view to age-grading specimens. Indeed, if nulliparous and parous flies can be distinguished, infection rates can be expressed in terms of parous flies only. This index has much greater epidemiological significance than crude infection rates of the total population examined.

Tables 3 and 4 summarize a selection of recent reports dealing with pro-

TABLE 3

Incidence of promastigote infections in Old World sandflies

Country	Number examined	Proportion infected	Author(s)
USSR and Turkmenian SSR	Not stated	4·6% *P. papatasi* 2·6% *P. caucasicus* 7·3% *S. 'arpaklensis'*	Neronov and Gunin (1971)
Iran	875[a]	3·6% *P. papatasi* 8·7% *P. caucasicus* 3·1% *P. ansarii*	Nadim *et al.* (1968a)
	1235[b]	3·0% *P. papatasi*	
	1203	8·7% *P. papatasi* 10·5% *P. caucasicus*	Nadim and Amini (1970)
	3012[a]	1·7% *P. alexandri* 0·2% *P. papatasi* 4·4% *S. sintoni*	Javadian and Mesghali (1974)
	443[b]	Nil	
Sudan	1171	1·9% *P. orientalis*	Heyneman (1963)
	1225	1·9% *P. orientalis*	Hoogstraal *et al.* (1962)
	10411	2·5% *P. orientalis*	Hoogstraal and Heyneman (1969)
Ethiopia	1216	0·3% *P. longipes*	Foster (1972)
	5848	3·7% *P. longipes*	Ashford *et al.* (1973a)
	37	5·4% *P. pedifer*	
Kenya	691	8·2% *P. pedifer* 1·2% *S. bedfordi*	Mutinga (1975)

[a] Collected from gerbil burrows.
[b] Collected at rest in houses.

mastigote infection rates in wild sandflies. The fly named *S. "arpaklensis"* by workers in USSR is, according to Lewis (1971), *S. sintoni*; according to Nadim *et al.* (1968a), *P. caucasicus* is a complex including *P. caucasicus* and *P. mongolensis*. Not all promastigotes isolated from sandflies were proved to be infective to mammals. Several of the infections discovered in Old World species, especially those in species of *Sergentomyia*, were suspected or shown to be of lizard origin. Of the five species of *Lutzomyia* found infected with promastigotes in Belize, only those isolated from *Lu. olmeca* gave rise to *L. mexicana mexicana* lesions when inoculated into golden hamsters. The nature and origins of infections in the other four *Lutzomyia* species remain unknown.

Adler (1964) commented on the remarkably high infection rates in Panamanian sandflies (Johnson *et al.*, 1963). Additional information explaining these high rates will be discussed later.

D. SANDFLIES AS HOSTS FOR TRYPANOSOMATIDS OTHER THAN *LEISHMANIA* INFECTIVE TO MAN

Leishmania of lizards are included here, though there is evidence (Adler, 1964) that some of the parasites infective to lizards can cause cryptic and transient infections in mammals, including man.

1. *Lizard* Leishmania *in phlebotomines*

Adler and Theodor (1957), after reviewing information about sandflies as vectors of lizard *Leishmania*, stated that the parasites developed in the hind gut of the insects but this opinion was modified (Adler, 1964) as a result of observations on the development of *L. adleri* in *S. clydei*. Localization of promastigotes in the mid gut need not indicate that the parasites are of mammalian origin. The females of *S. sintoni*, though usually feeding on poikilothermic vertebrates, also feed on gerbils and, occasionally, man. In Kenya, *S. clydei* feeds on lizards, gerbils and, occasionally, man. Both species have been found with natural promastigote infections in the mid gut (Wijers and Minter, 1962; Lewis, 1971). Neither the position of the parasites in the gut, nor knowledge of the feeding habits of the flies, afford clues to the origins of the promastigotes. To identify promastigotes in Old World sandflies, infectivity tests and serological studies, like those described by Ponirovsky (1975), may be needed and it may also be necessary to study the effects of the isolated strains on experimentally infected sandflies (Alekseev *et al.*, 1975).

Garnham (1971a) listed five species—*P. papatasi, P. caucasicus, S. "arpaklensis", S. minuta* and *S. clydei*—as suspected insect hosts for lizard *Leishmania*. Nadim *et al.* (1968b) reported naturally infected *S. sintoni* in areas where the main rodent hosts for *Leishmania* were absent but many lizards were present; *Leishmania* was isolated from a lizard of the genus *Agama*. Hoogstraal and Heyneman (1969) suspected that *S. clydei* is the natural insect host of *L. hoogstraali* in Sudan. Mutinga (1975) concluded that infections found in *S. bedfordi* in Kenya were most likely of reptilian origin.

TABLE 4

Incidence of promastigote infections in New World sandflies

Country	Number examined	Proportion infected	Author(s)
Mexico	3462	5·9% *Lu. olmeca*	Biagi *et al.* (1965)
Belize	1238	Nil	Williams *et al.* (1965)
	2231	0·5% *Lu. olmeca*	Disney (1968)
		0·7% *Lu. permira*	
	13981	0·7% *Lu. olmeca*	Williams (1970)
		0·3% *Lu. permira*	
		0·2% *Lu. ovallesi*	
		0·1% *Lu. panamensis*	
		0·1% *Lu. cruciata*	
Costa Rica	324	14·2% *Lu. ylephiletor*	Zeledón and Alfaro (1973)
		3·4% *Lu. shannoni*	
Panama	5250	15·4% *Lu. trapidoi*	Johnson *et al.* (1963)
		9·4% *Lu. ylephiletor*	
		5·4% *Lu. shannoni*	
		5·0% *Lu. gomezi*	
		4·7% *Lu. sanguinaria*	
		1·9% *Lu. panamensis* (*Lu. longipalpis*)	
	1748	0·3% *Lu. panamensis*	Christensen *et al.* (1969)
	575	Nil	Christensen *et al.* (1972)
	1435	0·6% *Lu. trapidoi*	Christensen and Herrer (1973)
		0·4% *Lu. panamensis*	

Trinidad	1954	1·0% *Lu. flaviscutellata*	Tikasingh (1975)
Surinam	Not stated	(12 *Lu. anduzei*)	Wijers and Linger (1966)
Brazil			
Pará	2706	0·3% *Lu. flaviscutellata*	Lainson and Shaw (1968)
	2701	0·2% *Lu. wellcomei*	Lainson *et al.* (1973)
		1·1% *Lu. paraensis*	
		0·8% *Lu. amazonensis*	
	9670	0·8% *Lu. flaviscutellata*	Ward *et al.* (1973)
Pará/Amapá	1010	7·3% *Lu. anduzei*	Lainson *et al.* (1976)
Bahia	1528	Nil	Sherlock and Guitton (1969)
Minas Gerais	1001	Nil	Williams and Falcão[a]
São Paulo	1612	0·6% *Lu. intermedia*	Forattini *et al.* (1972)
		0·6% *Lu. pessoai*	

[a] Unpublished observations.

Lizard leishmaniasis does not create problems in studying leishmaniases in the Americas. The only known species (*L. henrici*) was described from Martinique. Phlebotomines are not abundant in the Caribbean Islands (Fairchild and Hertig, 1948; Fairchild and Trapido, 1950) and only one species has been reported from Martinique (Floch and Abonnenc, 1952). Although this species probably feeds on reptiles, it does not seem to be a particularly abundant insect. Because *L. henrici* occurs as promastigotes in the cloaca of lizards, coprophagous insects which are later eaten by lizards are the more likely vehicles of transmission.

2. *Sandflies as hosts for* Trypanosoma *spp.*

Table 5 lists the 10 species of *Trypanosoma* known to have Phlebotominae as their insect hosts. Johnson and Hertig (1970) reported that an unnamed trypanosome of the woolly opossum in Panama developed in laboratory-reared *Lu. sanguinaria.*

Of the trypanosomes associated with sandflies, only *T. phyllotis* and *T. leonidasdeanei* infect mammals. Both belong to the subgenus *Megatrypanum*, the most primitive subgenus of mammalian trypanosomes (Hoare, 1972) which has many affinities with trypanosomes of reptiles and amphibians (Hoare, 1964). The other species associated with Phlebotominae are parasites of reptiles (six species) or amphibians (two species).

Although widely separated geographically, the blood forms of *T. bocagei* and *T. bufophlebotomi* are almost identical and their development and behaviour in the sandfly gut are similar (Ayala, 1971). The development of *T. bufophlebotomi* in the Californian sandfly *Lu. vexator occidentis* is remarkably similar to that of *T. boueti* in the Ethiopian fly *S. bedfordi* (Ashford *et al.*, 1973b). The parasites pass through the same sequence of flagellate forms (trypomastigote, amastigote, sphaeromastigote, promastigote, epimastigote); initial multiplication occurs in the stomach but, as the residues of the blood meal are evacuated, the parasites pass into the hind gut and become concentrated in the posterior part of the intestine just in front of the rectal ampulla. The development of other trypanosomes associated with sandflies is, in general, similar to that of *T. bufophlebotomi* and *T. boueti. T. gerrhonoti* and *T. scelopori*, however, initially multiply in the stomach of *Lu. vexator occidentis* and then migrate forwards to become concentrated in the cardiac stomach.

When Anderson and Ayala (1968) first described the development of *T. bufophlebotomi*, they commented that its promastigotes in the sandfly stomach could be mistaken, in a focus of leishmaniasis, for promastigotes of *Leishmania.* Ayala (1971) reported that the forms of *T. bufophlebotomi* in the stomach of *Lu. vexator occidentis* do not conform with any of the definitions of Hoare and Wallace (1966) and are intermediate between promastigotes and epimastigotes. Some of the epimastigotes illustrated by Ashford *et al.* (1973b) were similar to the "promastigotes" of Ayala (1971). The trypanosome of the woolly opossum also transforms into promastigotes in the gut of *Lu. sanguinaria* (Johnson and Hertig, 1970). With these exceptions, *Trypanosoma* spp. in

the sandfly gut are usually morphologically distinguishable from the promastigotes of *Leishmania.*

Theodor (1965) considered sandflies whose males bear five spines on the dististyle to be the most primitive of the subfamily Phlebotominae. It is interesting to note, therefore, that the males of most of the sandflies which act as hosts for *Trypanosoma* spp. possess this primitive characteristic. The primitive characteristics of both the parasites and their insect hosts, the morphological and developmental similarities between the trypanosomes themselves and the wide geographical separation of the known examples, suggest that the *Trypanosoma*/Phlebotominae association is of ancient evolutionary origin and that the present examples represent relicts of what could have been a widespread and, perhaps, common situation.

Sandfly/trypanosome associations might well be commoner and more widespread, however, than existing records show. Epimastigotes were found in *S. garnhami* in Kenya (Lewis and Minter, 1960) and trypomastigotes were recorded in *S. garnhami* in Sudan (Hoogstraal and Dietlein, 1963; Hoogstraal and Heyneman, 1969). Correa (1964) found epimastigotes and trypomastigotes in *Lu. trapidoi* and *Lu. sanguinaria* in Panama. Christensen and Herrer (1973), also working in Panama, reported such infections from *Lu. micropyga, Lu. panamensis* and *Lu. sanguinaria* and referred to flagellates of undetermined morphology in *Lu. gomezi, Lu. panamensis, Lu. sanguinaria* and *Lu. trapidoi.* In Belize, Williams (1970) found trypomastigotes in *Lu. shannoni* and speculated that they may have originated from ant-eaters, in which Lainson (1965) detected *T. legeri*, a member of the subgenus *Megatrypanum* for which the insect host remains unknown (Wells, 1976). Flagellates other than promastigotes of *Leishmania* have been found in *Lu. infraspinosa, Lu. davisi* (called *Lu. rooti* in the original paper) and *Lu. sallesi* collected in the State of Pará, Brazil (Shaw and Lainson, 1972). Sherlock and Pessoa (1966) found flagellates in *Lu. micropyga* (a misidentification of *Lu. schreiberi*) collected in the State of Bahia. Recently, Williams and Falcão (unpublished results) have discovered epimastigotes in *Lu. sallesi* and *Lu. edwardsi* collected in the State of Minas Gerais.

Speculations on the evolution of Trypanosomatidae are outside the scope of this review, but the apparently ancient association between large species of *Trypanosoma* and Phlebotominae could represent an unsuccessful experiment in trypanosome evolution or the connecting link between the genera *Trypanosoma* and *Leishmania.*

3. *Sandflies as hosts for* Endotrypanum

The evidence that sandflies are the normal insect hosts for *Endotrypanum* has resulted chiefly from studies in Panama on *E. schaudinni* in the two-toed sloth *Choloepus hoffmanni* and in several species of *Lutzomyia* (Shaw, 1964, 1969; Christensen and Herrer, 1976).

Shaw (1964, 1969) captured all haematophagous insects attracted to feed on a two-toed sloth placed on a 30ft (9 m) high tree platform. Phlebotomines accounted for almost 80% of the blood-sucking insects captured. The species

TABLE 5

Trypanosoma *spp. associated with Phlebotominae*

Parasite	Vertebrate host	Sandfly host	Country	Author(s)
T. phlebotomi	*Hemidactylus frenatus*	*S. babu*	India	Short and Swaminath (1931)[a]
T. platydactyli	*Tarantola mauretanica*	*S. minuta*	Malta	Adler and Theodor (1935)[a]
T. bocagei	*Bufo bufo*	*S. squamirostris*	China	Feng and Chung (1940)[a]
T. phyllotis	*Phyllotis darwinii*	*Lu. noguchii*	Peru	Herrer (1942)
T. leonidasdeanei	*Saccopteryx bilineata*	*Lu. vespertilionis*	Costa Rica	Zeledón and Rosabal (1969)
T. leonidasdeanei	*Saccopteryx bilineata*[b]	*Lu. vespertilionis*	Panama	Christensen and Herrer (1975)

T. leonidasdeanei(?)	*Carollia subruta*[c]	*Lu. beltrani* (Belize form)	Belize	Williams (1976)
T. bufophlebotomi	*Bufo boreas*	*Lu. vexator occidentis*	USA (California)	Ayala (1970); Anderson and Ayala (1968)
T. scelopori	*Sceloporus occidentalis*	*Lu. vexator occidentis*	USA (California)	Ayala (1970)
T. gerrhonoti	*Gerrhonotis multicarinatus*	*Lu. vexator occidentis*	USA (California)	Ayala and McKay (1971)
T. thecadactyli	*Thecadactylus rapicaudus*	*Lu. trinidadensis*	Panama	Christensen and Telford (1972)
T. boueti	*Mabuya striata*	*S. bedfordi*	Ethiopia	Ashford *et al.* (1973b)

[a] Quoted from Adler and Theodor (1957)
[b] Possibly also in *Peropteryx macrotis*.
[c] Probably also in *Glossophaga soricina* and *Artibeus lituratus*.

collected on the sloth were *Lu. trapidoi, Lu. ylephiletor, Lu. sanguinaria* and *Lu. gomezi*; specimens of each of these species were found infected with flagellates when they were collected after feeding on the host. Two out of eight laboratory-reared *Lu. sanguinaria* became infected when fed on *C. hoffmanni* harbouring *E. schaudinni*. In one of these flies, dissected six days after the blood meal, haptomonads and nectomonads were found in the hind gut. Shaw (1969) suggested that new vertebrate hosts became infected by the contaminative method.

Christensen and Herrer (1976) fed laboratory-reared *Lu. sanguinaria, Lu. gomezi* and *Lu. trapidoi* on *C. hoffmanni* infected with *E. schaudinni* and dissected specimens 1–23 days after the blood meal. Infection rates were significantly higher in *Lu. gomezi* than in *Lu. sanguinaria*; high infection rates were recorded in only one of the batches of *Lu. trapidoi* used in the trials. In the sandfly gut, the parasites were rounded or oval promastigotes attached to the gut wall and exhibiting little activity, but unattached active promastigotes were seen in the gut lumen of many flies. Flagellates were recorded in all parts of the gut between the cardiac stomach and the rectal ampulla, with highest concentrations in the pylorus. Although no flagellate was found anterior to the cardia, Christensen and Herrer (1976) thought it more likely that sandflies transmit *E. schaudinni* by inoculation than by contamination.

The two-toed sloth *C. hoffmanni* is the main forest host for *L. braziliensis* in Panama (Herrer *et al.*, 1973) as well as being the principal vertebrate host for *E. schaudinni*. The insect hosts of *L. braziliensis* in Panama are *Lu. trapidoi, Lu. panamensis, Lu. sanguinaria, Lu. gomezi* and *Lu. ylephiletor;* females of three of these species, possibly of all, are suitable insect hosts for *E. schaudinni*. In their insect hosts, both *L. braziliensis* and *E. schaudinni* multiply as promastigotes and concentrate in the same part of the sandfly gut, the pylorus. Because strains of promastigotes isolated from *Lu. trapidoi* and *Lu. gomezi* were closely related to a *Leishmania* from porcupines (Schneider, 1968), at least two of the five commonest mammal-biting sandflies of Panama are insect hosts for *L. hertigi*. In their studies on natural infection rates in Panamanian sandflies, Johnson *et al.* (1963) were probably dealing with two distinct strains of *L. braziliensis* (Schneider and Hertig, 1966), *L. hertigi*, known to infect only one vertebrate, a porcupine (Schneider, 1968; Herrer, 1971), *E. schaudinni* (Shaw, 1964, 1969; Christensen and Herrer, 1976), and a monoxenous parasite (Wallace and Hertig, 1968). This complexity of host–parasite relationships between Phlebotominae and Trypanosomatidae is, at present, only known to exist in Panama but could well be discovered in other foci of human leishmaniases.

4. *Monoxenous flagellate parasites of sandflies*

From studies on the ultrastructure of parasites isolated from Panamanian sandflies, Wallace and Hertig (1968) concluded that a strain isolated from *Lu. sanguinaria* was probably a species of *Crithidia*. An unusual morphology and growth pattern were noted when the fly was dissected. No monoxenous flagellate of Phlebotominae was listed by Wallace (1966) and the parasite from *Lu. sanguinaria* is the only authentic record.

Strangways-Dixon and Lainson (1962, 1966) suggested that promastigotes found, in very small numbers, in one *Lu. ovallesi* and one *Lu. cruciata* might have been monoxenous parasites. Promastigote infections in *Lu. permira, Lu. cruciata, Lu. panamensis* and *Lu. ovallesi* collected in Belize (Disney, 1968; Williams, 1970) and which failed to infect hamsters (and, in some cases, man) might also have been purely insect parasites. However, from what is now known about the host–parasite relationships of Phlebotominae and Trypanosomatidae in Panama, failure to infect golden hamsters or man with promastigotes from sandflies can no longer be accepted as proof that the organisms are monoxenous insect parasites.

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References

Adler, S. (1956). The relationship between *Leishmania* sp. and their sandfly vectors. *Revista Brasileira de Malariologia e Doenças Tropicais* **8**, 29–32.

Adler, S. (1964). *Leishmania. Advances in Parasitology* **2**, 35–96.

Adler, S. and Gunders, A. E. (1964). Immunity to *Leishmania mexicana* following spontaneous recovery from oriental sore. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **58**, 274–277.

Adler, S. and Theodor, O. (1957). Transmission of disease agents by phlebotomine sandflies. *Annual Review of Entomology* **2**, 203–226.

Adler, S., Foner, A. and Montiglo, B. (1966). The relationship between human and animal strains of *Leishmania* from the Sudan. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **60**, 380–386.

Alekseev, A. N., Saf'janova, V. M. and Karapetian, A. B. (1975). [On the viability of sandflies (Diptera, Psychodidae, Phlebotominae) after infection with promastigotes of different species of *Leishmania*.] *Parazitologiya* **9**, 271–277. [In Russian].

Anderson, J. R. and Ayala, S. C. (1968). Trypanosome transmitted by *Phlebotomus:* first report from the Americas. *Science*, New York **161**, 1023–1024.

Anonymous. (1971). Introduction [to special issue on leishmaniasis]. *Bulletin of the World Health Organization* **44**, 471.

Ashford, R. W., Bray, M. A., Hutchinson, M. P. and Bray, R. S. (1973a). The epidemiology of cutaneous leishmaniasis in Ethiopia. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **67**, 568–601.

Ashford, R. W., Bray, M. A. and Foster, W. A. (1973b). Observations on *Trypanosoma boueti* (Protozoa) parasitic in the skink *Mabuya striata* (Reptilia) and the sandfly *Sergentomyia bedfordi* in Ethiopia. *Journal of Zoology* **171**, 285–292.

Ayala, S. C. (1970). Two new trypanosomes from California toads and lizards. *Journal of Protozoology* **17**, 370–373.

Ayala, S. C. (1971). Trypanosomes in wild California sandflies, and extrinsic stages of *Trypanosoma bufophlebotomi. Journal of Protozoology* **18**, 433–436.

Ayala, S. C. and McKay, J. G. (1971). *Trypanosoma gerrhonoti* n. sp., and extrinsic development of lizard trypanosomes in California sandflies. *Journal of Protozoology* **18**, 430–433.

Belova, E. M. (1971). Reptiles and their importance in the epidemiology of leishmaniasis. *Bulletin of the World Health Organization* **44**, 553–560.

Biagi F., F., Biagi, A. M. de B. and Beltran H., F. (1965). *Phlebotomus flaviscutellatus*, transmisor natural de *Leishmania mexicana*. *Prensa Médicina Méxicana* **30**, 267–272

Biagi F., F., Biagi, A. M. de B. and Beltran H., F. (1966). *Phlebotomus flaviscutellatus*, transmisor natural de *Leishmania mexicana*. *Revista de Investigación en Salud Publica* **26**, 9–16.

Biagi F., F., Biagi, A. M. de B. and Beltran H., F. (1967). *Phlebotomus flaviscutellatus*, transmisor natural de *Leishmania mexicana*. *Revista do Instituto de Medicina Tropical de São Paulo* **9**, 18–26.

Bray, R. S. (1969). Studies on the immunology and serology of leishmaniasis: VI. Search for antigenic variations in single strains of *Leishmania* spp. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **63**, 378–382.

Bray, R. S. (1972). Leishmaniasis in the Old World. *British Medical Bulletin* **28**, 39–43.

Bray, R. S. (1974). *Leishmania*. *Annual Review of Microbiology* **28**, 189–217.

Bray, R. S. and Bryceson, A. D. M. (1969). Studies on the immunology and serology of leishmaniasis: VII. The identity of strains of *Leishmania* from Ethiopian diffuse cutaneous leishmaniasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **63**, 524–527.

Bray, R. S. and Lainson, R. (1965). The immunology and serology of leishmaniasis. I. The fluorescent antibody staining technique. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **59**, 535–544.

Bray, R. S. and Lainson, R. (1966). The immunology and serology of leishmaniasis. IV. Results of Ouchterlony double diffusion tests. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **60**, 605–609.

Bray, R. S. and Lainson, R. (1967). Studies on the immunology and serology of leishmaniasis. V. The use of particles as vehicles in passive agglutination tests. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **61**, 490–505.

Bray, R. S. and Mumford, F. (1967). On the maintenance of strains of *Leishmania* from the Guianas. *Journal of Tropical Medicine and Hygiene* **70**, 23–24.

Bray, R. S. and Rahim, G. A. F. (1969). Studies on the immunology and serology of leishmaniasis. VII. Serotypes of *Leishmania tropica*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **63**, 383–387.

Bray, R. S., Ashford, R. W., Mukherjee, A. M. and Sen Gupta, P. C. (1973a). Studies on the immunology and serology of leishmaniasis. IX. Serological investigation of the parasites of Indian kala-azar and Indian post-kala-azar dermal leishmaniasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **67**, 125–129.

Bray, R. S., Ashford, R. W. and Bray, M. A. (1973b). The parasite causing cutaneous leishmaniasis in Ethiopia. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **67**, 345–348.

Brazil, R. P. (1974). A new *Leishmania* of the *L. mexicana* complex. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **68**, 340.

Brazil, R. P. (1975). Observation on *Leishmania enriettii*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **69**, 365.

Cahill, K. M. (1964). Leishmaniasis in the Sudan Republic XXI. Infection in American personnel. *American Journal of Tropical Medicine and Hygiene* **13**, 794–799.

Chance, M. L. (1972). DNA base composition differences between species of

Leishmania. Transactions of the Royal Society of Tropical Medicine and Hygiene **66**, 352.

Chance, M. L. and Gardener, P. J. (1965). Biochemical and morphological differentiation within the genus *Leishmania. Transactions of the Royal Society of Tropical Medicine and Hygiene* **69**, 9.

Chance, M. L., Peters, W. and Griffiths, H. W. (1973). A comparative study of DNA in the genus *Leishmania. Transactions of the Royal Society of Tropical Medicine and Hygiene* **67**, 24–25.

Chance, M. L., Peters, W. and Shchory, L. (1974). Biochemical taxonomy of *Leishmania*. I: Observations on DNA. *Annals of Tropical Medicine and Parasitology* **68**, 307–316.

Christensen, H. A. and Herrer, A. (1973). Attractiveness of sentinel animals to vectors of leishmaniasis in Panama. *American Journal of Tropical Medicine and Hygiene* **22**, 578–584.

Christensen, H. A. and Herrer, A. (1975). *Lutzomyia vespertilionis* (Diptera: Psychodidae): potential vector of chiropteran trypanosomes in Panama. *Journal of Medical Entomology* **12**, 477–478.

Christensen, H. A. and Herrer, A. (1976). Neotropical sandflies (Diptera: Psychodidae), invertebrate hosts of *Endotrypanum schaudinni* (Kinetoplastida: Trypanosomatidae). *Journal of Medical Entomology* **13**, 299–303.

Christensen, H. A. and Telford, S. R. Jr. (1972). *Trypanosoma thecadactyli* sp. n. from forest geckoes in Panama and its development in the sandfly *Lutzomyia trinidadensis* (Newstead) (Diptera, Psychodidae). *Journal of Protozoology* **19**, 403–406.

Christensen, H. A., Herrer, A. and Telford, S. R. Jr. (1969). *Leishmania braziliensis* s. lat. isolated from *Lutzomyia panamensis* in Panama. *Journal of Parasitology* **55**, 1090–1091.

Christensen, H. A., Herrer, A. and Telford, S. R. Jr. (1972). Enzootic cutaneous leishmaniasis in Eastern Panama. II. Entomological investigations. *Annals of Tropical Medicine and Parasitology* **66**, 55–66.

Coelho, M. de V. (1964). Pesquisas recentes sobre a transmissão da leishmaniose tegumentar americana. *Revista Brasileira de Malariologia e Doenças Tropicais* **16**, 13–18.

Coelho, M. de V. (1966). "Desenvolvimento de espécies do gênero *Leishmania em* espécies brasileiras de flebótomos do gênero *Lutzomyia* França, 1924" Instituto Nacional de Endemias Rurais, Belo Horizonte (mimeographed).

Coelho, M. de V. and Falcão, A. R. (1962). Transmissão experimental de *Leishmania braziliensis*. 2. Transmissão da amostra *mexicana* por picada de *Phlebotomus longipalpis* e de *Phlebotomus renei. Revista do Instituto de Medicina Tropical de São Paulo* **4**, 220–224.

Coelho, M. de V., Falcão, A. R. and Falcão, A. L. (1967a). Desenvolvimento de espécies do gênero *Leishmania* em espécies brasileiras de flebótomos do gênero *Lutzomyia* França, 1924. I. Evolução de *L. braziliensis* em flebótomos. *Revista do Instituto de Medicina Tropical de São Paulo* **9**, 177–191.

Coelho, M. de V., Falcão, A. R. and Falcão, A. L. (1967b). Desenvolvimento de espécies do gênero *Leishmania* em espécies brasileiras de flebótomos do gênero *Lutzomyia* França, 1924. II. Ciclo vital de *L. tropica* em *L. longipalpis* e *L. renei. Revista do Instituto de Medicina Tropical de São Paulo* **9**, 192–196.

Coelho, M. de V., Falcão, A. R. and Falcão, A. L. (1967c). Desenvolvimento de espécies do gênero *Leishmania* em espécies brasileiras de flebótomos do gênero *Lutzomyia* França, 1924, III. Ciclo vital de *L. mexicana* em *L. longipalpis* e *L. renei. Revista do Instituto de Medicina Tropical de São Paulo* **9**, 299–303.

Coelho, M. de V., Falcão, A. R. and Falcão, A. L. (1967d). Desenvolvimento de espécies do gênero *Leishmania* em espécies brasileiras de flebótomos do gênero *Lutzomyia* França, 1924. IV. Ciclo vital de *L. donovani* em *L. longipalpis* e *L. renei*. *Revista do Instituto de Medicina Tropical de São Paulo* **9**, 361–366.

Coelho, M. de V., Falcão, A. R. and Falcão, A. L. (1967e). Desenvolvimento de espécies do gênero *Leishmania* em espécies brasileiras de flebótomos do gênero *Lutzomyia* França, 1924. V. Infetividade de leptomonas evoluindo no flebótomo e experiências de transmissão de leishmanioses. *Revista do Instituto de Medicina Tropical de São Paulo* **9**, 367–373.

Correa A., M. D. (1964). Tripanosomas encontrados en especies panameñas de *Phlebotomus*. *Archivos Medicos Panameños* **13**, 183–194.

Davis, N. T. (1967). Leishmaniasis in the Sudan Republic. 28. Anatomical studies on *Phlebotomus orientalis* Parrot and *P. papatasi* Scopoli (Diptera: Psychodidae). *Journal of Medical Entomology* **4**, 50–65.

Disney, R. H. L. (1968). Observations on a zoonosis: leishmaniasis in British Honduras. *Journal of Applied Ecology* **5**, 1–59.

Fairchild, G. B. (1949). A new fly related to *Phlebotomus* from Panama (Diptera, Psychodidae). *Proceedings of the Entomological Society of Washington* **51**, 81–84.

Fairchild, G. B. and Hertig, M. (1948). Notes on the *Phlebotomus* of Panama (Diptera, Psychodidae) IV. *P. atroclavatus* Knab, *P. cayennensis* Floch and Abonnenc, *P. chiapanensis* Dampf and some related forms from the West Indies and Mexico. *Annals of the Entomological Society of America* **41**, 455–467.

Fairchild, G. B. and Trapido, H. (1950). The West Indian species of *Phlebotomus* (Dipt., Psychodidae). *Annals of the Entomological Society of America* **43**, 405–417.

Floch, H. and Abonnenc, E. (1952). "Faune de l'Union française XIV. Diptères Phlébotomes de la Guyane et des Antilles française." Office de la Recherche Scientifique Outre-Mer, Paris.

Forattini, O. P. (1971). Sobre a classificação de subfamília Phlebotominae nas Américas (Diptera, Psychodidae). *Papéis Avulsos de Zoologia*, São Paulo **24**, 93–111.

Forattini, O. P. (1973). "Entomologia Médica", vol. 4. Edgard Blücher and Universidade de São Paulo, São Paulo.

Forattini, O. P., Pattoli, D. B. G., Rabello, E. X. and Ferreira, O. A. (1972). Infecção natural de flebotomineos em foco enzoótico de leishmaniose tegumentar no Estado de São Paulo, Brasil. *Revista de Saúde Pública* **6**, 431–433.

Foster, W. A. (1972). Studies on leishmaniasis in Ethiopia. V: Distribution, infections and assessment of vector potential of *Phlebotomus longipes* (Diptera: Psychodidae). *Annals of Tropical Medicine and Parasitology* **66**, 445–455.

Gardener, P. J. (1974). Pellicle-associated structures in the amastigote stage of *Trypanosoma cruzi* and *Leishmania* species. *Annals of Tropical Medicine and Parasitology* **68**, 167–176.

Gardener, P. J. and Howells, R. E. (1972). Isoenzyme variation in leishmanial parasites. *Journal of Protozoology* **19**, (Supplement), 47.

Gardener, P. J., Chance, M. L. and Peters, W. (1974). Biochemical taxonomy of *Leishmania*. II: Electrophoretic variation of malate dehydrogenase. *Annals of Tropical Medicine and Parasitology* **68**, 317–325.

Garnham, P. C. C. (1971a). The genus *Leishmania*. *Bulletin of the World Health Organization* **44**, 477–489.

Garnham, P. C. C. (1971b). American leishmaniasis. *Bulletin of the World Health Organization* **44**, 521–527.

Gunders, A. E., Foner, A. and Montilio, B. (1968). Identification of *Leishmania* species isolated from rodents in Israel. *Nature*, London **219**, 85–86.

Herrer, A. (1942). *Trypanosoma phyllotis* n. sp. e infeciones asocialas en una titiru, el *Phlebotomus noguchii. Revista de Medicina Experimental,* Lima **1**, 40–55.

Herrer, A. (1971). *Leishmania hertigi* sp. n., from the tropical porcupine, *Coendou rothschildi* Thomas. *Journal of Parasitology* **57**, 626–629.

Herrer, A., Thatcher, V. E. and Johnson, C. M. (1966). Natural infections of *Leishmania* and trypanosomes demonstrated by skin culture. *Journal of Parasitology* **52**, 954–957.

Herrer, A., Christensen, H. A. and Beumer, R. J. (1973). Reservoir hosts of cutaneous leishmaniasis among Panamanian forest mammals. *American Journal of Tropical Medicine and Hygiene* **22**, 585–591.

Hertig, M. (1948). A new genus of bloodsucking psychodid from Peru (Diptera: Psychodidae). *Annals of the Entomological Society of America* **41**, 8–16.

Hertig, M. and McConnell, E. (1963). Experimental infection of Panamanian *Phlebotomus* sandflies with *Leishmania. Experimental Parasitology* **14**, 92–106.

Heyneman, D. (1963). Leishmaniasis in the Sudan Republic 12. Comparison of experimental *Leishmania donovani* infections in *Phlebotomus papatasi* (Diptera: Psychodidae) with natural infections found in man-baited *P. orientalis* captured in a kala-azar endemic region of the Sudan. *American Journal of Tropical Medicine and Hygiene* **12**, 725–740.

Heyneman, D. (1971). Immunology of leishmaniasis. *Bulletin of the World Health Organization* **44**, 499–514.

Hoare, C. A. (1964). Morphological and taxonomic studies on mammalian trypanosomes. X. Revision of the systematics. *Journal of Protozoology* **11**, 200–207.

Hoare, C. A. (1972). "The trypanosomes of mammals." Blackwell Scientific Publications, Oxford and Edinburgh.

Hoare, C. A. and Wallace, F. G. (1966). Developmental stages of trypanosomatid flagellates, a new terminology. *Nature*, London **212**, 1385–1386.

Hoogstraal, H. and Dietlein, D. R. (1963). Leishmaniasis in the Sudan Republic 9. Ecological relationships of sandfly species and *Leishmania* infections. *American Journal of Tropical Medicine and Hygiene* **12**, 165–174.

Hoogstraal, H. and Heyneman, D. (1969). Leishmaniasis in the Sudan Republic 30. Final epidemiologic report. *American Journal of Tropical Medicine and Hygiene* **18**, 1091–1210.

Hoogstraal, H., Dietlein, D. R. and Heyneman, D. (1962). Leishmaniasis in the Sudan Republic 4. Preliminary observations on man-biting sandflies (Psychodidae: *Phlebotomus*) in certain Upper Nile endemic areas. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **56**, 411–422.

Javadian, E. and Mesghali, A. (1974). Studies on cutaneous leishmaniasis in Khuzestan, Iran. Part I. The leptomonad infection of sandflies. *Bulletin de la Société de Pathologie Exotique* **67**, 513–516.

Johnson, P. T. and Hertig, M. (1970). Behaviour of *Leishmania* in Panamanian phlebotomine sandflies fed on infected animals. *Experimental Parasitology* **27**, 281–300.

Johnson, P. T., McConnell, E. and Hertig, M. (1963). Natural infection of leptomonad flagellates in Panamanian *Phlebotomus* sandflies. *Experimental Parasitology* **14**, 107–122.

Kilgour, V., Gardener, P. J., Godfrey, D. G. and Peters, W. (1974). Demonstration of electrophoretic variation of two aminotransferases in *Leishmania*. *Annals of Tropical Medicine and Parasitology* **68**, 245–246.

Killick-Kendrick, R. (1976). Designations of strains of parasitic Protozoa. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **70**, 352.

Killick-Kendrick, R., Molyneux, D. H. and Ashford, R. W. (1974). *Leishmania* in phlebotomid sandflies. I. Modifications of the flagellum associated with attachment to the mid-gut and oesophageal valve of the sandfly. *Proceedings of the Royal Society*, London, Series B **187**, 409–419.

Killick-Kendrick, R., Sinden, R. E. and Leaney, A. J. (1975). Scanning electron microscopy of *Leishmania* from midguts of experimentally infected sandflies. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **69**, 432.

Kirk, R. (1950). Experimental differentiation of *Leishmania*. *Parasitology* **40**, 58–59.

Lainson, R. (1965). Parasitological studies in British Honduras I. A parasite resembling *Trypanosoma* (*Schizotrypanum*) *cruzi* in the coati, *Nasua nasua* (Carnivora, Procyonidae), and a note on *Trypanosoma legeri* from the anteater, *Tamandua tetradactyla* (Edentata). *Annals of Tropical Medicine and Parasitology* **59**, 37–42.

Lainson, R. and Shaw, J. J. (1968). Leishmaniasis in Brazil: I. Observations on enzootic rodent leishmaniasis—incrimination of *Lutzomyia flavescutellata* (Mangabeira) as the vector in the lower Amazonian basin. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **62**, 385–395.

Lainson, R and Shaw, J. J. (1969a). Some reservoir-hosts of *Leishmania* in wild animals of Mato Grosso State, Brazil. Two distinct strains of parasites isolated from man and rodents. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **63**, 408–409.

Lainson, R. and Shaw, J. J. (1969b). Leishmaniasis in Brazil: III. Cutaneous leishmaniasis in an opossum, *Marmosa murina* (Marsupialia, Didelphidae) from the lower Amazon region. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **63**, 738–740.

Lainson, R. and Shaw, J. J. (1970). Leishmaniasis in Brazil: V. Studies on the epidemiology of cutaneous leishmaniasis in Mato Grosso State, and observations on two distinct strains of *Leishmania* isolated from man and forest animals. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **64**, 654–667.

Lainson, R. and Shaw, J. J. (1971). Epidemiological considerations of the leishmanias with particular reference to the New World. *In* "Ecology and Physiology of Parasites" (A. M. Fallis, ed.), pp. 21–57. University of Toronto Press, Toronto.

Lainson, R. and Shaw, J. J. (1972). Leishmaniasis of the New World: taxonomic problems. *British Medical Bulletin* **28**, 44–48.

Lainson, R. and Shaw, J. J. (1973). Leishmanias and leishmaniasis of the New World, with particular reference to Brazil. *Bulletin of the Pan American Health Organization* **7**, 1–19.

Lainson, R. and Shaw, J. J. (1974). Las leishmanias y la leishmaniasis del nuevo mundo, con particular referencia al Brasil. *Boletín de la Oficina Sanitaria Panamericana* **76**, 93–114.

Lainson, R. and Strangways-Dixon, J. (1963). *Leishmania mexicana:* the epidemiology of dermal leishmaniasis in British Honduras. I. The human disease. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **57**, 242–265.

Lainson, R. and Strangways-Dixon, J. (1964). The epidemiology of dermal

leishmaniasis in British Honduras: Part II. Reservoir hosts of *Leishmania mexicana* among the forest rodents. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **58**, 136–153.

Lainson, R., Shaw, J. J. and Lins, Z. C. (1969). Leishmaniasis in Brazil: IV. The fox, *Cerdocyon thous* (L.) as a reservoir of *Leishmania donovani* in Pará State, Brazil. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **63**, 741–745.

Lainson, R., Shaw, J. J., Ward, R. D. and Fraiha, H. (1973). Leishmaniasis in Brazil: IX. Considerations of the *Leishmania braziliensis* complex—importance of sandflies of the genus *Psychodopygus* (Mangabeira) in the transmission of *L. braziliensis braziliensis* in North Brazil. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **67**, 184–196.

Lainson, R., Ward, R. D. and Shaw, J. J. (1976). Cutaneous leishmaniasis in North Brazil: *Lutzomyia anduzei* as a major vector. *Transactions of the Royal Society of Tropical Medicine and Parasitology* **70**, 171–172.

Lemma, A., Foster, W. A., Gemetchu, T., Preston, P. M., Bryceson, A. and Minter, D. M. (1969). Studies on leishmaniasis in Ethiopia I. Preliminary investigations into the epidemiology of cutaneous leishmaniasis in the highlands. *Annals of Tropical Medicine and Hygiene* **63**, 455–472.

Lewis, D. J. (1971). Phlebotomid sandflies. *Bulletin of the World Health Organization* **44**, 535–551.

Lewis, D. J. (1974). The biology of Phlebotominae in relation to leishmaniasis. *Annual Review of Entomology* **19**, 363–384.

Lewis, D. J. and Minter, D. M. (1960). Internal structural changes in some African Phlebotominae. *Annals of Tropical Medicine and Parasitology* **54**, 351–365.

Lumsden, W. H. R. (1974). Biochemical taxonomy of *Leishmania*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **68**, 74–75.

Lumsden, W. H. R. (1976). Designation of strains of parasitic Protozoa. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **70**, 352.

Luz, E., Giovannoni, M. and Borba, A. M. (1966/67). Infecção de *Lutzomyia monticula* por *Leishmania enriettii*. *Anais da Faculdade de Medicina da Universidade Federal de Paraná* **9/10**, 121–128.

Lysenko, A. Ja. (1971). Distribution of leishmaniasis in the Old World. *Bulletin of the World Health Organization* **44**, 515–520.

Mangabeira Filho, O. (1942). 8ª contribuição ao estudo dos *Flebotomus* (Diptera: Psychodidae). *Flebotomus* (*Brumptomyia*) *avellari* Costa Lima, 1932. *Memórias do Instituto Oswaldo Cruz* **37**, 225–240.

Manson-Bahr, P. E. C. (1955). A primary skin lesion in visceral leishmaniasis. *Nature*, London **175**, 433–434.

Manson-Bahr, P. E. C. (1971). Leishmaniasis. *International Review of Tropical Medicine* **4**, 123–140.

Marsden, P. D. and Nonata, R. R. (1975). Mucocutaneous leishmaniasis—a review of clinical aspects. *Revista da Sociedade Brasileira de Medicina Tropical* **9**, 309–326.

Martins, A. V., Williams, P. and Falcão, A. L. (In press). "American sandflies (Diptera: Psychodidae—Phlebotominae)." Academia Brasileira de Ciências; Rio de Janeiro.

Mattock, N. M. (1975). Electrophoretic investigation of some dehydrogenases in *Leishmania* amastigotes. *Annals of Tropical Medicine and Parasitology* **69**, 345–348.

McConnell, E. (1964). Leishmaniasis in the Sudan Republic 17. Infections in

Phlebotomus orientalis Parrot (Diptera: Psychodidae) after feeding on a human kala-azar patient. *Journal of Tropical Medicine and Hygiene* **67**, 88–89.

McMillan, B. (1965). Leishmaniasis in the Sudan Republic 22. *Leishmania hoogstraali* sp. n. in the gecko. *Journal of Parasitology* **51**, 336–339.

Molyneux, D. H., Killick-Kendrick, R and Ashford, R. W. (1975). *Leishmania* in phlebotomid sandflies III. The ultrastructure of *Leishmania mexicana amazonensis* in the midgut and pharynx of *Lutzomyia longipalpis*. *Proceedings of the Royal Society*, London, Series B **190**, 341–357.

Morales, N. M., Schaefer, F. W. III, Keller, S. J. and Meyer, R. R. (1972). Effects of intercalating dyes on the kinetoplast of *Leishmania tropica*. *Journal of Protozoology* **19**, (Supplement), 40–41.

Moškovskij, Š. D. and Dunhamina, N. N. (1971). Epidemiology of the leishmaniases: general considerations. *Bulletin of the World Health Organization* **44**, 529–524.

Moškovskij, Š. D. and Southgate, B. A. (1971). Clinical aspects of leishmaniasis with special reference to the USSR. *Bulletin of the World Health Organization* **44**, 491–497.

Mutinga, M. J. (1975). *Phlebotomus* fauna in the cutaneous leishmaniasis focus of Mt. Elgon, Kenya. *East African Medical Journal* **52**, 340–347.

Nadim, A. and Amini, H. (1970). Seasonal variations of natural leptomonad infection in sandflies of rodent burrows. *Tropical and Geographical Medicine* **22**, 476–478.

Nadim, A., Mesghali, A. and Amini, H. (1968a). The epidemiology of cutaneous leishmaniasis in the Isfahan Province of Iran III. The vector. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **62**, 543–549.

Nadim, A., Seyedi Rashti, M. A. and Mesghali, A. (1968b). On the nature of leptomonads found in *Sergentomyia sintoni* in Khorassan, Iran and their relation to lizard Leishmanias. *Journal of Tropical Medicine and Hygiene* **71**, 240.

Neronov, V. M. and Gunin, P. D. (1971). Structure of natural foci of zoonotic cutaneous leishmaniasis and its relationship to regional morphology. *Bulletin of the World Health Organization* **44**, 577–584.

Newton, B. A. (1976). Biochemical approaches to the taxonomy of kinetoplastid flagellates. *In* "Biology of the Kinetoplastida" (W. H. R. Lumsden and D. A. Evans, eds), vol. 1, pp. 405–434. Academic Press, London, New York and San Francisco.

Perfil'ev, P. P. (1966). "[Fauna of the USSR. Diptera, Volume III, No. 2 Phlebotomidae (Sandflies).]" Academy of Sciences of the USSR, Moscow. [In Russian: Translated into English by J. Salkind (1968). Israel Program for Scientific Translations, Jerusalem.]

Petriščeva, P. A. (1971). The natural focality of leishmaniasis in the USSR. *Bulletin of the World Health Organization* **44**, 567–576.

Ponirovsky, E. N. (1975). [A comparative study of some methods of *Leishmania* identificati on] *Parazitologiya* **9**, 139–141. [In Russian].

Rioux, J. A. and Golvan, Y. J. (1969). "Epidémiologie des leishmanioses dans le sud de la France." Monograph No. 37, Institut National de la Recherche médicale, University of Montpellier, Paris.

Saf'janova, V. M. (1966). [Serological comparison of leptomonad strains isolated from sandflies with *Leishmania tropica* and leptomonads of reptiles.] *Meditsinskaya Parazitologiya i Parazitarnye Bolezni* **35**, 686–695. [In Russian].

Saf'janova, V. M. (1971). Leishmaniasis control. *Bulletin of the World Health Organization* **44**, 561–566.

Sanyal, A. B. and Sen Gupta, P. D. (1967). Fine structure of *Leishmania* in dermal leishmanoid. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **61**, 211–216.

Schildkraut, C. L., Mandel, M., Levisohn, S., Smith-Sonneborn, J. E. and Marmur, J. (1962). Deoxyribonucleic acid base composition and taxonomy of some Protozoa. *Nature*, London **196**, 795–796.

Schneider, C. R. (1968). Immunodiffusion studies on a skin-inhabiting *Leishmania* from the tropical porcupine, *Coendou rothschildi* Thomas. *Journal of Parasitology* **54**, 638–639.

Schneider, C. R. and Hertig, M. (1966). Immunodiffusion reactions of Panamanian *Leishmania*. *Experimental Parasitology* **18**, 25–34.

Shaw, J. J. (1964). A possible vector of *Endotrypanum schaudinni* of the sloth *Choloepus hoffmanni* in Panama. *Nature*, London **201**, 447–448.

Shaw, J. J. (1969). "The haemoflagellates of sloths." (London School of Tropical Medicine and Hygiene, Memoir No. 13). H. K. Lewis, London.

Shaw, J. J. and Lainson, R. (1972). Leishmaniasis in Brazil: VI. Observations on the seasonal variations of *Lutzomyia flaviscutellata* in different types of forest and its relationship to enzootic rodent leishmaniasis (*Leishmania mexicana amazonensis*). *Transactions of the Royal Society of Tropical Medicine and Hygiene* **66**, 709–717.

Shaw, J. J. and Lainson, R. (1976). Leishmaniasis in Brazil: XI. Observations on the morphology of *Leishmania* of the *braziliensis* and *mexicana* complexes. *Journal of Tropical Medicine and Hygiene* **79**, 9–13.

Sherlock, I. A. and Guitton, N. (1969). Observações sobre calazar em Jacobina, Bahia III. Alguns dados sobre o *Phlebotomus longipalpis*, o principal transmissor. *Revista Brasileira de Malariologia e Doenfas Tropicais* **21**, 541–548.

Sherlock, I. A. and Pessoa, S. B. (1966). *Leptomonas* infetando naturalmente *Phlebotomus* em Salvador (Bahia, Brasil). *Revista Latinoamericana de Microbiología y Parasitología* **8**, 47–50.

Stauber, L. A. (1966). Characterization of strains of *Leishmania donovani*. *Experimental Parasitology* **18**, 1–11.

Strangways-Dixon, J. and Lainson, R. (1962). Dermal leishmaniasis in British Honduras: transmission of *L. braziliensis* by *Phlebotomus* species. *British Medical Journal* **1**, 297–299.

Strangways-Dixon, J. and Lainson, R. (1966). The epidemiology of dermal leishmaniasis in British Honduras. Part III. The transmission of *Leishmania mexicana* to man by *Phlebotomus pessoanus*, with observations on the development of the parasite in different species of *Phlebotomus*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **60**, 192–201.

Theodor, O. (1948). Classification of the Old World species of the subfamily Phlebotominae (Diptera, Psychodidae). *Bulletin of Entomological Research* **39**, 85–115.

Theodor, O. (1965). On the classification of American Phlebotominae. *Journal of Medical Entomology* **2**, 171–197.

Tikasingh, E. S. (1975). Observations on *Lutzomyia flaviscutellata* (Mangabeira) (Diptera: Psychodidae) a vector of enzootic leishmaniasis in Trinidad, West Indies. *Journal of Medical Entomology* **12**, 228–232.

Velasco, J. and Trapido, H. (1974). Two new phlebotomine sandflies from Bolivia, *Lutzomyia boliviana* n. sp. and *Warileya yungasi* n. sp. (Diptera: Psychodidae). *Journal of Medical Entomology* **11**, 433–436.

Vickerman, K. (1976). The diversity of the kinetoplastid flagellates. *In* "Biology of

the Kinetoplastida" (W. H. R. Lumsden and D. A. Evans, eds), vol. 1, pp. 1–34. Academic Press, London, New York and San Francisco.

Wallace, F. G. (1966). The trypanosomatid parasites of insects and arachnids. *Experimental Parasitology* **18**, 124–193.

Wallace, F. G. and Hertig, M. (1968). Ultrastructural comparison of promastigote flagellates (leptomonads) of wild-caught Panamanian *Phlebotomus*. *Journal of Parasitology* **54**, 606–612.

Ward, R. D., Lainson, R. and Shaw, J. J. (1973). Further evidence of the rôle of *Lutzomyia flaviscutellata* (Mangabeira) as the vector of *Leishmania mexicana amazonensis* in Brazil. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **67**, 608–609.

Wells, E. A. (1976). Subgenus *Megatrypanum*. *In* "Biology of the Kinetoplastida" (W. H. R. Lumsden and D. A. Evans, eds), vol. 1, pp. 257–285. Academic Press, London, New York and San Francisco.

Wertheim, G., Foner, A. and Montilio, B. (1970). Changes in leptomonads of *Leishmania tropica* grown in media containing immune serum. *Nature*, London **226**, 267–269.

White, G. B. (1976). Zoological nomenclature in medical parasitology and entomology. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **70**, 166.

Wijers, D. J. B. and Linger, R. (1966). Man-biting sandflies in Surinam (Dutch Guiana): *Phlebotomus anduzei* as a possible vector of *Leishmania braziliensis*. *Annals of Tropical Medicine and Parasitology* **60**, 501–508.

Wijers, D. J. B. and Minter, D. M. (1962). Studies of the vector of kala-azar in Kenya I.—Entomological evidence. *Annals of Tropical Medicine and Parasitology* **56**, 462–472.

Williams, P. (1966). Experimental transmission of *Leishmania mexicana* by *Lutzomyia cruciata*. *Annals of Tropical Medicine and Parasitology* **60**, 365–372.

Williams, P. (1970). Phlebotomine sandflies and leishmaniasis in British Honduras (Belize). *Transactions of the Royal Society of Tropical Medicine and Hygiene* **64**, 317–368.

Williams, P. (1976). Flagellate infections in cave-dwelling sandflies (Diptera, Psychodidae) in Belize, Central America. *Bulletin of Entomological Research* **65**, 615–629.

Williams, P. Lewis, D. J. and Garnham, P. C. C. (1965). On dermal leishmaniasis in British Honduras. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **59**, 64–71.

Zeledón, R. and Alfaro, M. (1973). Isolation of *Leishmania braziliensis* from a Costa Rican sandfly and its possible use as a human vaccine. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **67**, 416–417.

Zeledón, R. and de Monge, E. (1967). Physiological studies on the culture form of four strains of *Leishmania braziliensis*. 1. Nitrogen content, substrate utilization, and effect of metabolic inhibitors on respiration and its relation to infectivity. *Journal of Parasitology* **53**, 937–945.

Zeledón, R. and Rosabal, R. (1969). *Trypanosoma leonidasdeanei* sp. nov. in insectivorous bats of Costa Rica. *Annals of Tropical Medicine and Parasitology* **63**, 221–228.

Zuckerman, A. (1975). Current status of the immunology of blood and tissue Protozoa. I. *Leishmania*. *Experimental Parasitology* **38**, 370–400.

Zuckerman, A. and Lainson, R. (1977). *Leishmania*. *In* "Parasitic Protozoa" (J. P. Kreier, ed.), vol. 1, pp. 57–133. Academic Press, New York, San Francisco and London.

The Sarcosporidia (Protozoa, Sporozoa): Life Cycle and Fine Structure

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I. Introduction

In the year 1843 Miescher observed, for the first time, cysts containing large numbers of banana-shaped Protozoa within the muscles of mice. Until 1882 these cysts were termed Miescher's tubes, Rainey's bodies or psorosperms. Because of the typical localization of the cysts within muscles Balbiani (1882) proposed the name Sarcosporidia. Today such cysts have been seen in reptiles, birds and many groups of mammals including man, monkeys and whales, thus indicating a broad host range and worldwide distribution (Babudieri, 1932; Kalyakin and Zasukhin, 1975; Mehlhorn *et al.*, 1976). The position of the Sarcosporidia within the zoological system remained doubtful, for their life cycles were unknown. Electron microscopy of the cyst stages and comparison with sporozoa led to the suggestion that there might be coccidian-like life cycles in Sarcosporidia (Sénaud, 1967; Mehlhorn and Scholtyseck, 1973). This suggestion was supported and stimulated by the finding of such a life cycle for *Toxoplasma gondii*, which also forms cysts and the fine structure of which was known to be very similar to that of *Sarcocystis* (Work and Hutchison, 1969; Overdulve, 1970; Frenkel *et al.*, 1970; Sheffield and Melton,

* Supported by DFG.

1970; Piekarski and Witte, 1970). The final proof of the coccidian-like nature of the Sarcosporidia was achieved by a series of transmission experiments with cysts from sheep, cattle and swine, in which a high prevalence had been reported as well as in several species of wild animals. In these experiments the sarcocysts within the muscles of the above-mentioned three species were shown to be developmental stages of already known coccidians of man, cat and dog (Rommel *et al.*, 1972; Heydorn and Rommel, 1972a,b; Rommel and Heydorn, 1972). Already in 1891 the latter had been described as *Isospora bigemina* Stiles and *I. hominis* Railliet and Lucet, but were thought to be strictly host specific. Now it became evident that these authors had observed only the stages within the final host, whereas asexual cyst formation always took place within the muscles of the intermediate host, in which they were described as sarcocysts.

These findings were confirmed by a number of authors in the following years (Fayer and Leek, 1973; Mahrt, 1973; Vershinin, 1973; Fayer and Johnson, 1973, 1974; Mehlhorn and Scholtyseck, 1974; Ford, 1974; Munday and Corbould, 1974; Markus *et al.*, 1974) and it became possible to ascertain the life cycles of other Sarcosporidia (Rzepczyk, 1974; Zaman and Colley, 1975; Rommel and Geisel, 1975; Janitschke *et al.*, 1976; Powell and McCarley, 1975; Tadros and Laarman, 1975; Černá, 1976; Černá and Loučková, 1976; Ruiz and Frenkel, 1976) as well as of the related organisms *Frenkelia* (Rommel and Krampitz, 1975) and *Besnoitia* (Wallace and Frenkel, 1975). At the same time the known life cycles were studied intensively using pure infecting materials (Mehlhorn *et al.*, 1975a,b,c,d; Heydorn *et al.*, 1975a,b; Gestrich *et al.*, 1975a,b; Munday *et al.*, 1975; Heydorn and Gestrich, 1976). Thus, there have been up to now such a large number of studies considering the life cycle and cytology, that a review seemed necessary.

II. General Considerations

Coccidia are characterized by a life cycle comprising three different successive phases. An asexual multiplication (*schizogony* = *merogony*) is followed by sexual differentiation (*gamogony*), both within the host cells, as well as fertilization and oocyst formation. Another asexual multiplication (*sporogony*) occurs inside the oocyst wall, leading finally to the formation of sporocysts containing the infectious sporozoites. The number of sporocysts and sporozoites formed within each oocyst is characteristic of the genus. Thus in *Isospora*, belonging to the family Eimeriidae, two sporocysts each containing four sporozoites are present in an oocyst excreted with the faeces. Originally it was thought that an oocyst of Eimeriidae could infect only one species of host, so that development was strictly *monoxenous* (= *monoecious*). In this systematic group an alternation of generations in different hosts (= *heteroxenous* development) was known to occur only in the genus *Aggregata*, where schizogony was observed exclusively in the crab and gamogony and sporogony in the cuttlefish (Levine, 1973; Pellérdy, 1974). On the other hand two-host cycles are typical of other coccidia like some adeleids, most of the Haemosporida and Piroplasmida (Grell, 1973; Levine, 1973; Schein, 1975; Mehlhorn

et al., 1975e). In such cases those hosts in which the sexual cycle proceeds are termed *final hosts*, whereas the others, where asexual development occurs, are described as *intermediate hosts*. *Toxoplasma gondii* was the first of the eimerian group to be shown recently to have a heteroxenous life cycle (reviewed by Frenkel, 1974). The alternation of hosts, however, is *facultative* in this life cycle because the final host (cat) and a variety of intermediate hosts can be infected by *Toxoplasma* cysts as well as by oocysts from the cat's faeces. In all *Sarcocystis* species studied up to now (Table 1) the alternation of hosts turned out to be *obligatory* (Fig. 1)* and the significance of carnivorism in the transmission became clear. The life cycle involves two vertebrate hosts: the "prey" (i.e. herbivores, omnivores) and a "predator" (i.e. carnivorous animals), with schizogony taking place in the "prey" (= intermediate host) and sexual reproduction of the parasite restricted to the intestine of the "predator" (= final host). Originally it was thought that each animal species was parasitized as intermediate host by only one sarcosporidian species and that the different cysts seen within the muscles were developmental stages of one cyst type. Therefore the morphologically different cysts observed in one intermediate host species were given a common species name, e.g. *Sarcocystis tenella* in sheep, *S. miescheriana* in swine and *S. fusiformis* in cattle, By recent transmission experiments, however, it was shown that several *Sarcocystis* species may form cysts in the same intermediate host (Fig. 2). Thus three different types of cysts have been demonstrated in cattle, two in sheep, two in swine, two in rats, two in rhesus monkeys and at least two in mice (Rommel *et al.*, 1974; Heydorn *et al.*, 1974; Ford, 1974; Munday and Rickard, 1974; Mehlhorn *et al.*, 1975 a-d, 1976, 1977; Gestrich *et al.*, 1975a,b; Rzepczyk and Scholtyseck, 1976; Zaman and Colley, 1975; Černá, 1976; Ruiz and Frenkel, 1976). Therefore a new nomenclature of the Sarcosporidia was proposed by Heydorn and colleagues (1975c) using the combination of *intermediate* and *final host* respectively as the new species name (Table 1).†

III. Development in the Intermediate Host (Schizogony)

The development of Sarcosporidia within an intermediate host, which had ingested oocysts and sporocysts (Figs 3–5), comprises two different phases: a phase of extraintestinal multiplication (Fig. 1, 1) is followed by cyst formation mostly in musculature (Fig. 1, 2).

A. Extraintestinal Schizogony

In sheep and cattle parasites were observed beginning on the 15th day after the ingestion of oocysts and sporocysts, whereas in rats and pigs they were already seen on the 4th to 9th days post infection (p.i.) (Fayer and Johnson,

* All figures are grouped at the end of this chapter.

† The current International Code of Zoological Nomenclature (ed. 2, 1964) obliges us to retain the names *S. fusiformis* and *S. tenella* for the species more logically named *S. bovifelis* and *S. ovifelis* respectively. (Since this Chapter was written, the nomenclature of *Sarcocystis* has been critically reviewed by Dubey (1976) and Levine (1977)—*Editors.*)

TABLE 1

List of all Sarcosporidia of which the whole life cycle is known

Species		Intermediate host	Final host	Size of sporocysts (μm)	Pathogenicity	Prepatent period (days)	Patent period (weeks)	References
old name	new name							
S. fusiformis	*S. bovihominis*	cattle	man, baboon, rhesus monkey	9·3 × 14·7	—	9–10	> 6	Rommel and Heydorn, 1972 Heydorn *et al.*, 1975c Heydorn *et al.*, 1976
	S. bovicanis	cattle	dog, coyote, red fox, racoon, wolf	10·8 × 16·3	+	9–10	> 6	Heydorn *et al.* 1975c, Fayer and Johnson, 1973, 1974; Heydorn and Rommel, 1972 a,b
	S. fusiformis[a]	cattle	cat, feral cat	7·8 × 12·5	—	7–9	> 6	Heydorn *et al.*, 1975c Heydorn and Rommel, 1972a, b
S. tenella	*S. ovicanis*	sheep	dog	9·9 × 14·8	+	8–9	> 6	Munday *et al.*, 1975 Heydorn *et al.*, 1975c
	S. tenella[b]	sheep	cat	8·1 × 12.4	—	11–14	> 6	Heydorn *et al.*, 1975c
S. miescheriana	*S. suihominis*	pig	man	9·3 × 12·6	+	10	> 6	Rommel *et al.*, 1974 Heydorn, 1977
	S. suicanis	pig	dog, fox, wolf	9·6 × 12·6	/	9–10	> 6	Rommel *et al.*, 1974
S. bertrami	*S. equicanis*	horse	dog	10·0 × 15·2	/	8	3	Rommel and Geisel, 1975
S. orientalis[c]		rat (*Rattus norvegicus*)	python (*P. reticulatus*)	7·7 × 9·1	+	/	/	Zaman and Colley, 1975

S. sp.		rat (*Rattus fuscipes*)	python (*Morelia spilotes*)	6·6 × 9·6	/	/	/	Rzepczyk, 1974
S. sp.		vole (*Microtus arvalis*)	weasel (*Mustela nivalis*)	9 × 12	/	7	3	Tadros and Laarman, 1975
S. sp.		mouse	owl (*Tyto alba*)	/	+	/	/	Černá, 1976
S. sp.		vole (*Microtus arvalis*)	kestrel (*Falco tinnunculus*)	10 × 14	+	/	/	Černá and Loučková, 1976
S. muris		mouse	cat	8·5 × 10·3	+	5–11	12	Ruiz and Frenkel, 1976
S. sp.[d]		*Gazella granti* cat dog		9·3 × 13·2 10·6 × 16·2	/ /	21 11	76	Janitschke *et al.*, 1976
S. cuniculi		rabbit (*Oryctolagus cuniculus*)	cat	9·6 × 12·6	/	9–10	/	Tadros and Laarman, 1977
S. leporum		cottontail rabbit (*Sylvilagus floridanus*)	cat	9·4 × 13·6	/	10–25	1–7	Fayer and Kradel, 1977
S. moulei		goat	dog	/	/	8–9	> 6	Fischer, 1978

+ = present, − = not present, / = information not given.

[a] The name *S. bovifelis* would be more logical, but the International Code of Zoological Nomenclature requires retention of the name *S. fusiformis* for this species.

[b] The name *S. ovifelis* would be more logical, but the International Code of Zoological Nomenclature requires retention of the name *S. tenella* for this species.

[c] This name must be changed, for it was applied to a sarcocyst of *Capra sibirica* by Machulski and Miskaryan (1958).

[d] This probably represents two species.

1973, 1974; Munday *et al.*, 1975; Mehlhorn *et al.*, 1975b; Gestrich *et al.*, 1975a; Heydorn and Gestrich, 1976; Zaman and Colley, 1975; Heydorn, 1977). In mice free merozoites were noted on the 4th day p.i. (Černá, 1976) or on the 11th–17th days p.i. (Ruiz and Frenkel, 1976), indicating that there may be differences between the *Sarcocystis* species parasitizing the tissues of the same intermediate host. In each case however, large numbers of free merozoites and intracellular schizonts with a maximum of about 50 nuclei were found. These parasites were distributed throughout the tissues of the intermediate host, usually within endothelial cells of blood vessels or in close proximity to these vessels. In several species the liver was often the most heavily parasitized organ, and contained most of the mature schizonts that were observed. In rats no schizont was noted within the brain, whereas Munday *et al.* (1975), Fayer and Johnson (1973) and Heydorn (1977) reported such stages within the cerebrum of sheep, pigs and calves. There is clear evidence that besides the multiplication by schizogony, endodyogeny also occurs. In calves, sheep and rats merozoites were observed, the nucleus of which was dividing showing the typical features of endodyogeny (Fig. 1, 1b). The present results indicate that several generations of schizonts may follow each other, each being morphologically similar or not, but further detailed studies are required. About one month p.i. the parasites leave the endothelial system and evidently enter the muscle cells where they start a new development. The merozoites and schizonts of several sarcosporidia proved to be very pathogenic and thus of high economic importance.* Such intermediate hosts as sheep, cattle and voles became severely ill and died or were near death after being infected with sporocysts of *S. ovicanis, S. bovicanis* or *S.* sp. from kestrels (Fayer and Johnson, 1974; Munday *et al.*, 1975; Heydorn and Gestrich, 1976). The pathological effects of sarcosporidian schizogony still need intensive investigation.

B. CYST FORMATION

About 40 days after the ingestion of sporocysts the schizonts and free merozoites had completely disappeared from the tissues examined. Beginning on the 25th–30th days p.i., however, development of cysts was seen within syncytial muscle fibres and muscle cells (Figs 1, 2; 11a; 22). Munday *et al.* (1975) and Heydorn (1977) noticed an additional cyst forming within the ovine and porcine brain after infecting sheep and pigs with sporocysts from dogs.

1. *Cyst wall*

When a merozoite had penetrated a muscle fibre, cyst development always started from a typical parasitophorous vacuole (Figs 10, A; 11a), first described in intestinal epithelial cells by Scholtyseck and Piekarski (1965). The border of this vacuole was always a single unit membrane (Fig. 10, A). This membrane soon became strengthened by an underlying layer of osmiophilic

* It now seems very probable that "Dalmeny Disease" of cattle is acute sarcocystosis, with extraintestinal schizogony in various tissues.

material (Fig. 10, B). This complex formed the *primary cyst wall* and reached a thickness of about 20–100 nm in the Sarcosporidia already studied (Mehlhorn *et al.*, 1976, 1977). A characteristic feature of the primary cyst wall was the presence of irregularly distributed unthickened places about 40 nm in diameter which formed vesicle-like invaginations into the interior of the cyst (Fig. 10, C). In some *Sarcocystis* species these invaginations covered the whole surface of the cyst (Fig. 15, B), whereas in others they were restricted to the bases of eventual protrusions and/or the spaces between them (Fig. 12, B). These small vesicle-like invaginations probably represent absorptive areas giving rise to the vesicles seen in the interior of the cyst. Such a primary cyst wall has been seen in all species of *Sarcocystis* studied by electron microscopy, as well as, with slight variations, in *Toxoplasma* and *Frenkelia* cysts (Sénaud, 1967; Kepka and Scholtyseck, 1970; Scholtyseck *et al.*, 1974; Mehlhorn *et al.*, 1974). In *Besnoitia jellisoni* of mice and in globidial schizonts within the intestine of sheep, the unit membrane of the original parasitophorous vacuole remained unthickened even in mature cysts (Sheffield, 1968; Sénaud, 1969; Mehlhorn and Heydorn, 1976; Heydorn and Mehlhorn, 1976).

In some species the primary cyst wall may form characteristic protrusions, which are identical in all mature cysts of the same species wherever they are located (Figs 7, CW; 8, PA; 13, VW; 15, ZA; 16), e. g. the mature cysts of *S. bovihominis* always had large palisade-like protrusions with fibrillar or tubular elements in their interior, whereas no such protrusions were seen in mature cysts from whales, mice or rhesus monkeys (Figs 9, 16). Cysts with straight palisade-like protrusions, the largest of which may reach in some species 10 μm in length, appear by light microscopy to have a radially striated "thick wall", because of the close proximity of the protrusions to each other (Fig. 7, CW). Cysts, however, where no protrusions exist or the protrusions are folded over along the surface of the cyst as in *Sarcocystis* sp. from grackles or in *S. bovicanis,* appear by light microscopy to have a relatively thin wall, as the zone of the superficially folded protrusions is only seldom thicker than 1 μm (Fig. 6, CW). Within the protrusions fibrils, tubules or microtubules may be formed or not (dependent upon the species; cf. Figs. 14–16). Although the ultrastructure of the primary cyst wall with its variations and the presence or absence of fibrillar, tubular or microtubular elements is characteristic of each species, morphology alone cannot be used for absolute species differentiation, as a similar type of cyst wall is present in several known different *Sarcocystis* species. So the value of the primary cyst wall as a criterion of species identification is only relative, but it can be used to differentiate between the different cysts in sheep, cattle or mice after infections with oocysts and sporocysts from different final hosts. This also indicates that only the parasites determine the structure of the primary cyst wall and its underlying elements, because—if not—all cysts in the muscles of the same intermediate host, e.g. sheep, cattle, swine, rats or mice, would be identical (Table 2).

During growth of the cysts there was always development of an amorphous ground substance beneath the primary cyst wall (Fig. 10, B, C). This substance containing finer fibrils and small granules traversed the interior of the cyst to form septa lining numerous compartments containing the parasites (Figs 8,

TABLE 2

List of all sarcosporidian species which have been studied by electron microscopy

Species old name	Species new name	Intermediate/final host	Differentiations of the primary cyst wall (PH)	Presence of septa in cyst	Size of metrocytes (μm)	Length of the merozoites (μm) within cysts	References
S. fusiformis	*S. bovihominis*	cattle/man	numerous palisade-like protrusions (4–7 × 0·7 μm) containing numerous fibrils	+	6–7 × 4–5	7–9	Mehlhorn *et al.*, 1975a
	S. bovicanis	cattle/dog	few flattened protrusions without fibrils follow a course along the cyst's surface	+	7 × 5	10–14	Mehlhorn *et al.*, 1975b
	S. fusiformis[a]	cattle/cat	numerous palisade-like protrusions (3·8 – 5·5 × 1·5 μm) containing 200–300 fibrils	+	12–14 × 5–7	13–15	Gestrich *et al.*, 1975a
S. tenella	*S. ovicanis*	sheep/dog	numerous palisade-like protrusions (2–3·5 × 0·5–0·8 μm) without fibrils	+	8 × 7	14–17	Mehlhorn *et al.*, 1975c
	S. tenella[b]	sheep/cat	numerous cauliflower-like protrusions (1–4·5 μm) with numerous microtubules	+	15–20 × 8–10	12–15	Sénaud, 1967 Mehlhorn and Scholtyseck, 1973
S. miescheriana	*S. suihominis*	swine/man	numerous protrusions up to 14μm, originally straight but becoming folded; containing circles of filaments	+	9–12 × 4–6	15	Mehlhorn and Heydorn, 1977
	S. suicanis	wild pig/dog?	palisade-like protrusions, 2·5–3·5 μm, with filaments	+	12 × 6	14	Mehlhorn *et al.* 1976; Erber and Boch, 1976

S. miescheriana		swine/?	large, finger-like protrusions (8–10 × 0·7–6·8 μm) with numerous fibrils	+	/	8–12	Ludvík, 1960
S. miescheriana		wild boar/?	numerous, palisade-like protrusions (2·5–3·5 × 1·5 μm) containing about 70 fibrils at their periphery	+	5 × 3	6–8	Mehlhorn *et al.*, 1976
S. bertrami	*S. equicanis*	horse/dog	numerous long protrusions (2 × 0·4 μm) containing 40–70 fibrils	+	/	3·2–6·5	Göbel, 1976
S. orientalis		rat/python	villus-like protrusions (4 × 0·3–1·0 μm) containing microtubules	/	/	4·5–6·0	Zaman and Colley, 1975
Sarcocystis sp.		rat/python	type A with large protrusions (6·8–8·7 × 0·8–1·4 μm) containing fibrils	+	5·5 × 3·2	4·7	Rzepczyk and Scholtyseck, 1976
			type B without any protrusion	+	4·1 × 2·7	4·6	
Sarcocystis sp.		rat/?	square stone-like protrusions (6 × 4 μm) containing fine granules	+	/	6·7	Mehlhorn *et al.*, 1976
Sarcocystis sp.		mouse/cat	no large protrusions, wavy surface	+	/	12·5	Viles and Powell, 1976
Sarcocystis sp.		mouse/owl	without definite protrusions, wavy surface	+	4 × 3	5–9	Černá and Sénaud, 1976

TABLE 2 (*continued*)

Species old name	Species new name	Intermediate/final host	Differentiations of the primary cyst wall (PH)	Presence of septa in cyst	Size of metrocytes (μm)	Length of the merozoites (μm) within cysts	References
S. muris		mouse/cat	without definite protrusions,	+	/	/	Sheffield, Frenkel and Ruiz, 1976
Sarcocystis sp.		grackle/?	wave-like protrusions ($5 \times 0{\cdot}3$–$1{\cdot}2$ μm) with bulbous ends containing bundles of microtubules	+	/	5–6	Zeve *et al.* 1966, Simpson and Forester, 1973, Mehlhorn *et al.*, 1976
Sarcocystis sp.		hen/?	small palisade-like protrusions ($1{\cdot}5 \times 0{\cdot}5$ μm) with numerous fibrillar elements	+	6×5	10–11	Mehlhorn *et al.*, 1976
Sarcocystis		sperm whale/?	without definite protrusions, but extensively folded	+	4×3	5–6	Mehlhorn *et al.*, 1976
S. balaenopteralis		whale/?	small villus-like structures, but no definite protrusions	+	/	7–8	Akao, 1970
Sarcocystis sp.		rhesus monkey/?	without definite protrusions, wavy in appearance	+	5×6	6	Mehlhorn *et al.*, 1977
Sarcocystis sp.		baboon/?	without definite protrusions, heavily folded	+	7×4	6·4–9·0	Mehlhorn *et al.*, 1977
Sarcocystis sp.		tamarin monkey/?	numerous nipple-like papillae in the heavily folded PH, without definite protrusions	+	4×4	4–5	Mehlhorn *et al.*, 1977

+ = present, / = information not given.
# See note (*a*) on Table 1.

9, 14, 18; GS, SE). In some species these septa are so small that they are invisible by light microscopy (Figs 7, 8), whereas in others such as the macroscopically visible cysts of *S. tenella* they may reach a considerable thickness (Fig. 21; GS, SE). Such compartments are absent in *Toxoplasma* and *Besnoitia*, but present in *Frenkelia* (Scholtyseck *et al.*, 1974). These septa may give a greater stability to the cyst, which may even be situated in a working muscle of the host. The peripheral zone of ground substance is considerably thicker in those *Sarcocystis* species which have no or only very short cyst wall protrusions.

The whole development of the cysts of Sarcosporidia takes place within the interior of syncytial muscle fibres or muscle cells, which are always recognizable by the presence of muscle fibrils, even when the muscle fibres contain very old cysts and are thus completely filled (Figs 7, 8, 11a–d, 15; HC). Up to three microscopically small cysts were observed within a single muscle fibre. Also in the case of the large, ovoid cysts of *S. tenella* from sheep, often reaching a size of up to 1·5 cm, the cyst proper is situated within a very stretched but recognizable muscle fibre (Fig. 15, HC). Outside this parasitized muscle fibre there is always a surrounding thick layer of fibrillar material (Fig. 15, SH). This zone, which is at least 2–4 μm thick and which may be considered a *secondary cyst wall*, is composed of connective tissue. Such a secondary cyst wall is, according to our present knowledge, seen only in *S. tenella* and *Besnoitia jellisoni* and is not found in the other sarcosporidia (Mehlhorn *et al.*, 1976).

Considering the morphology of the sarcocysts from a variety of hosts as described to date and compared with other cyst-forming genera like *Toxoplasma* and *Besnoitia*, it may be stated that the cysts, although parasitizing quite different muscles, are constructed according to a common plan. Therefore in those species, where the life cycle is unknown, a similar type of life cycle may be expected. On the other hand, studies of early cyst development make it clear, that a real cyst* or a solid cyst wall as known in other protozoa, is not typically present in sarcosporidian muscle cysts. So we have for the cyst forming coccidia the following definitions:

Cyst: Transformed parasitophorous vacuole within a parasitized host cell (muscle fibre etc). In old cysts of *Toxoplasma* and *Frenkelia* the remainder of the host cell may completely disappear. The parasitophorous vacuole, or its periphery only, becomes filled with a granular ground substance of unknown origin.

Primary cyst wall: Thickened border (unit membrane plus underlying osmiophilic material) of the transformed parasitophorous vacuole, which may give rise to characteristic protrusions in the different species.

* Lainson (1958) defined a true cyst as an investing, protective or resistant structure formed wholly or partly from substances secreted from the body of the organism, as distinct from the normal limiting membrane of the living host cell. Although this definition is not fully applicable, if at all, to these "resting stages" of Sarcosporidia, *Toxoplasma, Besnoitia* and *Frenkelia*, as electron microscopic studies of their development have shown, we think that the term "cyst" can be used for them as they are functionally very similar to cysts as defined by Lainson.

Secondary cyst wall: Structure of fibrillar material of host origin which encloses the parasitized host cell. Only existing as far as is known in *Besnoitia* and *Sarcocystis tenella.*

Pseudocyst: Used of *Toxoplasma* to describe a host cell, with a parasitophorous vacuole containing several parasites in the acute proliferative phase of development. It is no "waiting stage" like the cysts, but a transitional one. Therefore this term should be discontinued in our opinion.

2. *Parasites*

Within the cysts of Sarcosporidia two types of parasites always occur: globular cells called *metrocytes* and more slender, often banana-shaped *merozoites*. The terms *tachyzoites* and *bradyzoites*, coined by Frenkel (1973, 1974), tend to give the impression of completely different stages and do not make it clear that development within the intermediate hosts occurs as schizogony throughout the whole life cycle of the Sarcosporidia. Furthermore there is no basic structural difference between the more or less banana-shaped stages in the cysts, termed *bradyzoites* by Frenkel, the infectious tissue-stages before cyst formation, termed *tachyzoites*, or the typical intestinal or non-intestinal merozoites of other coccidia. The term *metrocyte*, however, characterizes a special merozoite in young cysts. This stage, which becomes globular, is much less differentiated than the mature merozoite and reproduces very often by *endodyogeny* and was therefore called mother cell (= metrocyte) by Sénaud (1967). At the beginning of cyst formation only metrocytes were found within the young cysts. About two months p.i. the cyst contained both metrocytes and merozoites, whereas about three months p.i. only very few metrocytes were seen situated exclusively at the periphery of the cyst. At this stage the chamber-like hollows within the ground substance of the cyst were closely filled with long merozoites. From this it may be concluded, that three months after introduction of sporocysts from the intermediate host, the cysts were fully differentiated and ready for renewed transmission. Thus in several transmission experiments infectivity was noted only when old sarcocysts were used (Heydorn and Gestrich, 1976; Ruiz and Frenkel, 1976).

(a) *Fine structure of metrocytes.* The metrocytes are globular cells of different length in the Sarcosporidia. Thus in cysts of rats, they reach only 3–5 μm, whereas in the macroscopic cysts of sheep, they were up to 15–20 μm long (for references see Table 3). Apart from variations in length, the metrocytes of Sarcosporidia were structured according to a common plan. They were limited by a typical three layered coccidian pellicle consisting of three unit membranes (Figs 19, 21, 22; PE). The surface of the smaller metrocytes was often somewhat "wavy", whereas mainly in larger metrocytes of other species deep invaginations of the pellicle occurred (Figs 19, 21; IN). Several micropores, evidently small cytostomes, were present along the surface (Figs 19, 21; MP). At the apical pole a typical conoid and polar ring with 22 anchored microtubules, and a Golgi complex anterior to the nucleus, were observed in all species studied (Figs 19, 21). Rhoptries (paired organelles) and micronemes were rarely found, even in older metrocytes (Fig. 19; R, MN). The

nucleus had a spherical nucleolus consisting of granular and fibrillar zones. Chromosomal structures were seen in two different stages: large dense plaques (*condensed stage*; Fig. 22, CH) and small dense granules of 30–40 nm diameter, arranged spherically within the karyoplasm (Fig. 21, arrows). The nuclear pores showed the typical eight-fold symmetry known from other protozoa and numerous metazoa. The cytoplasm of the metrocytes appeared very electron-lucent and in some cases extremely vacuolated. By *endodyogeny*, two daughter cells are formed within these metrocytes. Clusters of these stages develop in this way, lying close together, often with "wavy" cell borders (Fig. 21, MC). After several endodyogenies, the developing cells became progressively similar to the later banana-shaped or slender merozoites found in older cysts, in which virtually no reproduction takes place. During endodyogeny the subpellicular microtubules were initially arranged around each pole of the dividing nucleus. Later the endoplasmic reticulum formed the two inner membranes of the pellicle around this "scaffold", which included conoid, two centrioles and two large vacuoles with a granular interior (= precursors of the rhoptries and micronemes). A bundle of about 20 microtubules was seen close to the nucleus within the developing daughter cells. For further details and a survey of literature see Heydorn *et al.* (1975a, b), Mehlhorn and Scholtyseck (1973) and Sénaud and Mehlhorn (1975).

(b) *Fine structure of the merozoites.* As is shown in Tables 2 and 3 and in the survey by Kalyakin and Zasukhin (1975), the merozoites of the *Sarcocystis* species differ considerably in size. The merozoites of *S. equicanis* (Göbel, 1976), *S. lindemanni* from man (Kutty and Dissanaike, 1975), *S.* sp. from tamarin monkeys (Mehlhorn *et al.*, 1977) and *S. platydactyli* from lizards (Sénaud, 1967) belong to the smallest group with a minimum length of about 3·5 μm, whereas those of *S. gracilis* from the tortoise or *S. tenella* may reach up to 18 μm (Lainson and Shaw, 1971; Sénaud, 1967; Mehlhorn and Scholtyseck, 1973). Apart from these differences in length and some variations in shape, the structure of the merozoites of Sarcosporidia is relatively uniform, as revealed up to now by electron microscopy. Thus it does not seem advisable to differentiate between several species by considering fine structure or size alone. Using scanning and transmission electron microscopy, and negative-staining, (Figs 17, 18, 23, 28), several typical structures were observed, as follows.

Pellicle: three layered as in other coccidia (Figs 20, 23; PE). The two inner layers were interrupted at the apical and the posterior pole, each forming a circular polar ring (Figs 20; P, PP; 23, 27, 28; P). In negatively stained material the inner layers of the pellicle appeared with a mesh-like pattern (Fig. 28; IM). At the posterior pole 11 rib-like elements were observed, evidently lying on the surface of the parasite. These ribs consisted of rows of granules, the function of which is unknown (Mehlhorn and Scholtyseck, 1973; Porchet-Henneré, 1975; D'Haese *et al.*, 1976).

Subpellicular microtubules: In all Sarcosporidia studied to date, 22 subpellicular microtubules were anchored at the anterior polar ring (Figs 26–28; ST).

TABLE 3

List of Sarcocysts in primates including man

	Sarcocysts described in	Cyst wall by light microscopy	Size of parasites within cysts (μm)	References
Old World	*Inuus* sp. (=*Macaca mulatta*)	with fine spikes	4–6	Ratzel, 1868
	Macaca mulatta	type A with 5–6 μm long, hair-like protrusions	9–14 × 3–4	Mandour, 1969
		type B with a thin, serrated appearing wall	7–9 × 2–3	
	Macaca mulatta	type A with 6·5 μm long, perpendicular protrusions	11 × 3	Karr and Wong, 1975
		type B without any protrusion	5 × 2	
	Macaca mulatta	wavy appearance, without protrusions	6	Mehlhorn *et al.*, 1976, 1977
	Macaca nemestrina *Macaca fascicularis* *Macaca arctoides*	bounded by a regular zone of 0·5–0·9 μm	4 × 1·8	Karr and Wong, 1975
	Macaca radiata	with long, hair-like processes	11 × 3	Karr and Wong, 1975
	Erythrocebus patas	with long protrusions	9–14 × 3–4	Mandour, 1964, 1969
	Nycticebus coucang	thin, without protrusions	/	Zaman, 1970
	Cercocebus atys	type A with hair-like processes type B without any processes	11 × 3 5 × 2	Karr and Wong, 1975

Old World	*Cercopithecus talapoin*	with long, perpendicular processes	11 × 3	Karr and Wong, 1975
	Cercopithecus mitis	with 5–6 μm long, hair-like processes	9–14 × 3–4	Mandour, 1969
	Papio sp.	/	/	Kim *et al.*, 1968
	Papio ursinus	/	/	McConnell *et al.*, 1974
	Papio papionis	without protrusions	5 × 2	Karr and Wong, 1975
	Papio cynocephalus	without protrusions, serrated appearance	6·4–9 × 2·3	Mehlhorn *et al.*, 1977
New World	*Saimiri sciureus*	without protrusions	5 × 2	Karr and Wong, 1975
	Saguinus nigricollis	/	/	Nelson *et al.*, 1966
	Saguinus (=*Oedipomidas*) *oedipus*	indistinct, without protrusions, fine constrictions	4–5 × 1·3–1·7	Mehlhorn *et al.*, 1977
Selected from about 30 cases	man (Russia)	/	4–5 × 1–2	Lindemann, 1863
	man (Sudan)	with 6–7 μm long protrusions	8	Kartulis, 1893
	man (Germany)	radially striated (=protrusions)	/	Lorenz, 1904
	man (Indonesia)	thin, without protrusions	4–5 × 1–2	Bonne and Soewandi, 1929
	man (England)	striated (=protrusions)	5 × 2	Hewitt, 1933
	man (England)	thin, without protrusions	6·5–7·3 × 1·1	Jeffrey, 1974
	man (Malaysia)	thin, without protrusions	3·2 × 1·2	Kutty and Dissanaike, 1975; Kutty *et al.*, 1975

Originally it was thought that these elements joined up with the posterior polar ring, but recently it was shown that in *S. tenella* they reached only to the middle of the parasite (D'Haese *et al.*, 1976). The function of these microtubules is not yet clear; they are probably concerned with motility.

Micropore: Normally one micropore was present in the anterior third of the merozoite, but occasionally two were seen to be functional, i.e. evidently ingesting small vesicles by large invaginations giving rise to vacuoles (Figs 20, 28; MP).

Conoid: This organelle was always present within the anterior polar ring and was sometimes protruding (Figs 20, 23, 26–28; C). It consisted of about 20 oblique microtubules surmounted by two rings with regular ornamentation (Fig. 27; C, RI). In the conoid's interior were two eccentric parallel microtubules which extended posteriorly for some distance into the adjacent cytoplasm (Fig. 28; MT).

Rhoptries: These club-shaped organelles were always present, in number up to 16 in several species (Figs 20, 23, 24, 26; R). Their function is doubtful.

Micronemes: Unlike the implication of their name, these structures appeared as "rice grains" in most species, and were very numerous, filling the whole anterior third of the merozoite (Figs 8, 20, 24; MN). In other sarcosporidia they may appear spherical or be distributed only at the margin in smaller numbers (Fig. 23). Their function, however, is not yet understood.

Mitochondrion: A single very long mitochondrion always occured, with typical tubular invaginations (Figs 20, 24, 25; MI).

Nucleus: The mostly globular nucleus limited by a typical nuclear membrane was always situated in the posterior half of the cell (Figs 20, 25; N). A spherical nucleolus was always prominent within the karyoplasm (Fig. 25, NU). Anterior to the nucleus was a Golgi complex.

Polysaccharide granules: Such granules were present in larger numbers in merozoites of younger cysts than in those of older cysts (Figs 8, 20, 24; A). These granules have been shown to decompose within vacuoles (Mehlhorn *et al.*, 1974).

Vacuoles: In several vacuoles, evidently originating from the invaginations of the micropores, digestion takes place, as was shown by the activity of acid phosphatase followed by alkaline phosphatase (Mehlhorn, 1975).

C. *Cytochemistry.* So far little is known concerning the cytochemistry of sarcosporidian cysts, although this subject seems favourable for study because of the relative easiness of obtaining large quantities of pure parasites. Attempts have been made to demonstrate at the ultrastructural level deoxyribo-

nucleic acid (DNA), ribonucleic acid (RNA) (Fig. 29), acid and alkaline phosphatase, adenosine triphosphatase (Fig. 30) and polysaccharides (Mehlhorn, 1975; Mehlhorn *et al.*, 1974; Sénaud and Mehlhorn, 1975). Recent results clearly indicate that the mature merozoites are "waiting stages" with reduced metabolism. This may be the reason why no inflammatory host reaction was observed around parasitized muscle fibres, although often completely filled by the cyst. However evidently cysts may degenerate, as described by Munday *et al.* (1975) for microscopical cysts of sheep. The lytic bacteria found within cysts of *S. tenella* may also lead to degeneration, for these bacteria destroy the pellicle of the parasites (Mehlhorn and Sénaud, 1975). Thus the inner compartments of the large cysts are completely empty, as reported in the first light microscope descriptions.

The significance of the so-called "sarcotoxin", present in extracts of the large cysts from sheep, is not yet understood. The toxicity to the rabbit of this material, first demonstrated by Pfeiffer in 1896 and reduced to its protein components by Sénaud *et al.* (1968), might be irrelevant to the host–parasite relationship in the sheep but perhaps it is of importance in the final host, the cat. Perhaps extracts of other sarcosporidian cysts contain similar protein fractions, which might be situated within the parasites and/or in the ground substance of the cyst.

Summarizing, it may be said of the Sarcosporidia, at least for the species studied, that *schizogony* (= merogony) has two phases. The first stage is a transient proliferative schizogony, mainly in vascular endothelial cells. During this time intermediate hosts may be killed by toxins evidently produced by the merozoites. This first phase is followed by a resting stage of schizogony within muscle fibres. The whole process takes about three months to produce cysts containing merozoites ready for transmission to the final host.

IV. Development in Final Hosts and Cell Cultures

As shown in Table 1, several *Sarcocystis* species may have more than one final host, but these are mostly closely related to each other. In the final hosts, schizogony never occurred whether they were fed *Sarcocystis* cysts within the musculature, or sporocysts, from final hosts (Heydorn and Rommel, 1972a, b; Fayer, 1974; Mehlhorn and Scholtyseck, 1974; Munday *et al.*, 1975; Zaman and Colley, 1975; Fischle, 1973; Rommel *et al.*, 1974; Ruiz and Frenkel, 1976; Černá and Loučková, 1976). Nor was schizogony observed when parasites from muscle cells were inoculated into cell cultures (Fayer, 1972; Vetterling *et al.*, 1973). Thus the merozoites from cysts evidently develop directly into gamonts and gametes *in vivo* as well as *in vitro*.

A. GAMOGONY

About one day after infection gamonts were seen within the cells of the intestine of the final host, lying within a clear parasitophorous vacuole (Fig. 1, 4). In the intestine of cats and dogs infected with cysts from cattle or sheep

the parasites were always situated subepithelially in the lamina propria of the mucosa and were especially numerous in the distal jejunum and proximal ileum. Zaman and Colley (1975), however, noted that, with *S. orientalis*, the epithelial cells of the rat's duodenum were highly infected. Ruiz and Frenkel (1976) reported that *S. muris* firstly penetrated the epithelium of the cat's intestine, usually through goblet cells. Later the evidently fertilized macrogametes moved to the level of the basement membrane or into the lamina propria. Perhaps this process is common in all Sarcosporidia, but further details are still needed. As in other coccidia, gamogony of the sarcosporidia proceeds as *oogamy*. Thus the nuclei of the growing female gamonts make no further divisions and the mature gametes, resembling the egg cells of metazoa, are then called *macrogametes*. In the male gamont, however, there is firstly a phase of mitotic nuclear divisions and later the actual formation of small gametes (= *microgametes*) begins (Scholtyseck *et al.*, 1971, 1972). Microgamonts and microgametes were seen in the *Sarcocystis* species only in very limited numbers and were identified with certainty only by means of electron microscopy (Vetterling *et al.*, 1973; Zaman and Colley, 1975). On the other hand, macrogametes were extremely numerous in the tissues examined.

Mature macrogametes appeared ovoid or spherical, attaining, in the species studied, a maximum diameter of about 8 μm and were characterized in light microscopical observations by large numbers of tiny periodic acid-Schiff (PAS) positive granules, first observed at the margin of the cytoplasm. Electron microscopy showed these macrogametes to be enclosed by a single membrane under which, at numerous places, remnants of the inner membranes of the original merozoites were seen. In the cytoplasm of these macrogametes numerous electron dense granules with increasing diameters up to 800 nm occurred. After fertilization these granules, which seemed identical with the PAS-positive ones seen by light microscopy, underwent disaggregation, and in this condition they appeared to consist of irregular osmiophilic masses with intervening electron-lucent spaces. These masses fused together to form a membrane-bound, homogeneous, osmiophilic layer, comprising the main part of the oocyst wall. Thus these granules are wall-forming bodies, known as *type 1* from other coccidia (Scholtyseck *et al.*, 1971; Mehlhorn, 1972a). Organelles similar to the wall-forming bodies of the *second type* were not observed in the Sarcosporidia.

Mature microgamonts reached about the same size as macrogamonts when containing at the maximum about 20–30 nuclei, arranged at the periphery where formation of microgametes took place as in other coccidia (Mehlhorn, 1972b; Scholtyseck *et al.*, 1972). After a condensation process in each nucleus, the electron-lucent part was separated from the osmiophilic one, which became the nucleus of the microgamete; the electron-lucent section remained a residual nucleus in the residual body. During this division a protrusion of the microgamont occurred comprising a tubular mitochondrion and growing flagella. Finally this protrusion (= new microgamete) was separated from the gamont and started movement. Thus the microgametes of Sarcosporidia consist of an elongated nucleus, a tubular mitochondrion, two free flagella and several additional microtubules under the enclosing unit membrane. In

S. orientalis the microgametes measured about 5 μm in length, thus being in the same size-range as those of most of the eimerians, whereas those of a species studied in cell cultures were only 3μm long (Vetterling *et al.*, 1973; Zaman and Colley, 1975). Concerning the cytology of the sexual development much more information is still needed. Fertilization has not yet been observed, but evidently starts on the earliest days, because often on the third day p.i. numerous oocysts were seen within the intestinal cells of the final host (Fig. 1; 5, 6).

The oocyst wall of the Sarcosporidia studied consisted of a relatively thin, dense outer layer and several underlying membranes (Figs 3, 31; OW). This thin outer layer was apparently formed by the breakdown of the dense granules of the mature macrogamete into smaller particles which fused to form this compact-appearing layer. In *Eimeria* species, however, two such layers are present which may also be thicker. This is perhaps the reason why the oocyst wall is very often broken in Sarcosporidia and thus the sporocysts are observed free within the faeces. The cytoplasm of the early oocyst (= *zygote*) was enclosed by one or two typical unit membranes (Mehlhorn and Scholtyseck, 1974; Vetterling *et al.*, 1973; Zaman and Colley, 1975) and contained large reserve granules (polysaccharides, lipids) with diameters up to 1·5μm (Fig. 31; A, L). In addition small spherical elements were found, forming a crystalline pattern (Fig. 31; CR). Osmiophilic bodies of 0·4 μm are probably responsible for the later formation of the sporocyst walls (Fig. 31; DG). The large nucleus was often situated at the periphery of the spherical unsporulated oocyst, which also possessed numerous tubular mitochondria. Occasionally micropores were still present as relics along the cytoplasmic membrane.

B. SPOROGONY

In all Sarcosporidia studied, sporulation took place within the parasitized intestinal cell of the final host (Fig. 31; HC), so that after the prepatent period mostly sporocysts with four sporozoites were found in the faeces, there being only a few oocysts containing two sporocysts (Figs 4,5). The first sporulation division resulted in an oocyst with two densely basophilic nuclei, at opposite poles of the cytoplasm. These nuclei then appeared to divide, accompanied by fission of the cytoplasmic mass into two portions. The result was the occurrence of two sporocyst progenitors, each with two polar U-shaped nuclei (Fig. 31). This second nuclear division was always followed by a third division and by separation of the cytoplasm within the sporocysts. Finally four sporozoites and a residual body consisting of granular material were present in each sporocyst (Figs 4, 5; S, RS). The unsporulated sporocysts were limited by a single osmiophilic layer and contained the same organelles as the unsporulated oocysts. Sporulated as well as unsporulated oocysts were always situated within a large electron-lucent parasitophorous vacuole (Fig. 31; PV). At this time the host cell consisted only of two remaining membranes: the outer cytoplasmic membrane (Fig. 31, HM) and the limiting membrane of the parasitophorous vacuole (Fig. 31, LP). The oocyst wall proved to be very fragile in the sporulated stage, so that mostly single, free sporocysts were

observed. The oocysts of all Sarcosporidia have no micropyle, and the absence of this and of a stieda-body and a substieda-body is a characteristic feature of these sporocysts.

Excretion of oocysts and sporocysts was not constant during the very long patent period. In the first days after the prepatent period only a few sporocysts were found within the faeces. Then for a short time the maximum shedding-rate occurred, whereas later few oocysts and sporocysts were excreted constantly. The long patent period, characteristic for these Sarcosporidia (Table 1), is not yet completely understood. Because there is no schizogonic process within the final host, it might be supposed that not all the merozoites from the muscle-cysts develop into gamonts at the same time. Perhaps these merozoites "wait" somewhere and develop only successively into gamonts and, after fertilization, into oocysts. Except for their size, the oocysts and sporocysts of Sarcosporidia look so similar that they cannot be distinguished where several species are present in the faeces of the final host. They show, however, quite different pathogenicity (Table 1). Several species (dog—cattle; dog—sheep) may cause acute infections in intermediate hosts, even with fatal results, whereas other species (man—cattle) have, even in high doses, no such effects. However, recent studies on *S. suihominis* by Heydorn (1977) and Mehlhorn and Heydorn (1977) showed that the final host (man) may also suffer from sarcosporidiosis; all volunteers who ate raw pig meat developed severe haemorhagic diarrhoea. The long-term effects of chronic infections, however, are not well studied. Thus, *Sarcocystis* has assumed a new medical and veterinary importance.

V. Sarcosporidiosis of Man and Other Primates

Omnivores like man and primates may be involved as final (Table 1) or intermediate (Table 3) hosts in the life cycles of several *Sarcocystis* species. As recently shown by Heydorn (1976), man may suffer severely as the final host of *Sarcocystis suihominis*, which forms cysts in muscle fibres of swine. Sarcocysts within muscles of man (as intermediate host) have been known since the first report of Lindemann (1963) and have been described from about 30 cases in a variety of countries, thus indicating a worldwide occurrence of this parasite (Jeffrey, 1974). All these cysts were found more or less occasionally and often no clinical manifestations were noted. This was due to the fact that normally only a local myositis occurred besides more general symptoms, which were thought to be caused by other diseases. The large number of cysts found in recent systematic surveys of various monkeys (Fig. 9), however, indicates that sarcocysts might be much more common in primates including man than had been thought (Kim *et al.*, 1968; McConnell *et al.*, 1974; Karr and Wong, 1975; Mehlhorn *et al.*, 1977). One *Sarcocystis* species of man (*S. lindemanni* Rivolta, 1878) and two of monkeys (*S. kortei* Castellani and Chalmers, 1909 and *S. nesbitti* Mandour, 1969) have been described. The morphological differences of the cysts studied (for references see Table 3) and the wide range of hosts, however, clearly indicate that there are almost certainly further *Sarcocystis* species parasitizing man and other primates. Up to now in no case

has the final host of such a species been found or even suggested. Thus in the near future investigations are needed into the transmission and eventual pathological effects of these parasites.

VI. Conclusions

The Sarcosporidia have adapted their life cycle to the "predator–prey" relationship existing between their hosts. Gamogony takes place in the "predator" (= *final host*), whereas schizogony (= merogony), leading to cyst formation, occurs exclusively in the "prey" (= *intermediate host*). Some carnivores may be final hosts of several *Sarcocystis* species and omnivores may be involved as intermediate as well as final hosts in the life cycles of different sarcosporidian species. Sporocysts or sporulated oocysts shed in the faeces of the final host must always be ingested by the intermediate host. The alternation of final and intermediate host is, according to our present knowledge, absolutely obligatory, thus clearly differing from that in the genus *Toxoplasma* (Frenkel, 1974). However, the question whether transport hosts may be involved in the sarcosporidian life cycle is not yet solved. Within such transport hosts no further development would occur, as was shown in mice infected with *Isospora felis* and *I. rivolta* (Frenkel and Dubey, 1972; Mehlhorn and Markus, 1976). Although the oocysts and sporocysts of the sarcosporidian life cycles are morphologically identical with those of the genus *Isospora*, and thus *Sarcocystis* has been classified as *Isospora* species (for references see Heydorn *et al.*, 1975c), there are four very important differences, as follows.

1. The Sarcosporidia have an obligatory two-host cycle (= heteroxenous).
2. In Sarcosporidia schizogony (= merogony) has two phases: an extra-intestinal multiplication followed by cyst formation mostly within the muscles of the intermediate host.
3. In Sarcosporidia no schizogonic multiplication occurs in the gut wall of the final host or in cell culture.
4. In Sarcosporidia oocysts are excreted fully sporulated; often single sporocysts are observed in the faeces of the final host because of the rupture of the extremely fine oocyst wall.

Considering these differences it seemed necessary to separate the Sarcosporidia from the genus *Isospora* and thus Heydorn *et al.* (1975c) proposed the retention of the generic name *Sarcocystis* Lankester, 1882 for all Sarcosporidia as defined above.

References

Akao, S. (1970). A new species of *Sarcocystis* parasitic in the whale *Balaenoptera borealis*. *Journal of Protozoology* **17**, 290–294.

Babudieri, R. (1932). I sarcosporidi e le sarcosporidiosi (studio monografico). *Archiv für Protistenkunde* **76**, 421–580.

Balbiani, G. (1882). Les Sporozoaires. *Journal de Micrographie* Paris **6**, 80–89.

Bonne, C. and Soewandi, R. (1929). *Sarcocystis* in man. *Geneeskundlijke Tijdschrift Nederland-Indie* **69**, 1104.

Castellani, A. and Chalmers, A. J. (1909). "Manual of tropical medicine." Baillière, Tindall and Co., London.

Černá, Z. (1976). *Sarcocystis* of mice (*Mus musculus*) acquired from oocysts isolated from the intestine of the owl (*Tyto alba*). *Proceedings of the XVth meeting of French speaking protozoologists*, Roscoff, 29.

Černá, Z. and Loučková. M. (1976). *Microtus arvalis* as the intermediate host of a coccidian from the kestrel (*Falco tinnunculus*). *Folia Parasitologica* (*Praha*) **23**, 110.

Černá, Z. and Sénaud, (1976). Étude ultrastructurale des xénoparasitomes sarcosporidiens obtenus après infestation expérimentale de la souris par *Isospora buteonis*. *Compte Rendu de la XV ème Réunion des Protistologues de la langue française*, Roscoff, 29.

Černá, Z. and Sénaud, J. (1977). Sur un type nouveau de multiplication asexuée d'une sarcosporidie dans le foie de la souris. *Compte Rendu de l'Academie des Sciences, Paris* **285**, 347–349.

D'Haese, J., Mehlhorn, H. and Peters, W. (1976). Charakteristische Strukturen von Coccidien im Negativkontrast. *Zeitschrift für Parasitenkunde* **50**, 198–199.

Dubey, J. P. (1976). A review of *Sarcocystis* of domestic animals and of other coccidia of cats and dogs. *Journal of the American Veterinary Association* **169**, 1061–1078.

Erber, M. and Boch, J. (1976). Untersuchungen über Sarkosporidien des Schwarzwildes. Sporocystenausscheidung durch Hund, Fuchs und Wolf. *Berlin-Münchner tierärztliche Wochenschrift* **89**, 449–450.

Fayer, R. (1972). Gamogony of *Sarcocystis* sp. in cell culture. *Science* **175**, 65–67.

Fayer, R. (1974). Development of *Sarcocystis fusiformis* in the small intestine of dogs. *Journal of Parasitology* **60**, 660–665.

Fayer, R. and Johnson, A. J. (1973). Development of *Sarcocystis fusiformis* in calves infected with sporocysts from dogs. *Journal of Parasitology* **59**, 1135–1137.

Fayer, R. and Johnson, A. J. (1974). *Sarcocystis fusiformis:* development of cysts in calves infected with sporocysts from dogs. *Proceedings of the Helminthological Society of Washington* **41**, 105–108.

Fayer, R. and Kradel, D. (1977). *Sarcocystis leporum* in cottontail rabbits and its transmission to carnivores. *Journal of Wildlife Diseases* **13**, 170–173.

Fayer, R. and Leek, R. G. (1973). Excystation of *Sarcocystis fusiformis* sporocysts from dogs. *Proceedings of the Helminthological Society of Washington* **40**, 294–296.

Fischer, G. (1978). Über die Entwicklung einer vom Hund übertragenen Sarkosporidienart in der Ziege. *Proceedings of 8. Tagung der deutschen Gesellschaft für Parasitologie in Freiburg*, p. 72.

Fischle, B. (1973). Üntersuchungen über die Möglichkeit einer Sporocystenausscheidung bei Katze und Hund nach Verfütterung von *Sarcocystis tenella*- und *Sarcocystis fusiformis*-Sporozysten. *Dissertation in Veterinary Medicine* Frein Universität, Berlin.

Ford, G. E. (1974). Prey–predator transmission in the epizootiology of ovine sarcosporidiosis. *Australian Veterinary Journal* **50**, 38–39.

Frenkel, J. K. (1973). Toxoplasmosis: parasite life cycle, pathology, and immunology. *In* "The coccidia" (D. M. Hammond and P. L. Long, eds), University Park Press, Baltimore.

Frenkel, J. K. (1974). Advances in the biology of Sporozoa. *Zeitschrift für Parasitenkunde* **45**, 125–162.

Frenkel, J. K. and Dubey, J. P. (1972). Rodents as vectors for feline coccidia, *Isospora felis* and *I. rivolta*. *Journal of infectious Diseases* **125**, 69–72.

Frenkel, J. K., Dubey, J. P. and Miller, N. L. (1970). *Toxoplasma gondii:* fecal stages identified as coccidian oocysts. *Science* **167**, 893–896.

Gestrich, R., Mehlhorn, H. and Heydorn, A. O. (1975a). Licht- und elektronenmikroskopische Untersuchungen an Cysten von *Sarcocystis fusiformis* in der Muskulatur von Kälbern nach experimenteller Infektion mit Oocysten und Sporocysten der grossen Form von *Isospora bigemina* der Katze. *Zentralblatt für Bakteriologie Parasitenkunde, Infektionskrankheiten und Hygiene, I. Abteilung, Originale, Reihe* **233**, 161–276.

Gestrich, R., Heydorn, A. O. and Baysu, N. (1975b). Beiträge zum Lebenszyklus der Sarkosporidien. VI. Untersuchungen zur Artendifferenzierung bei *Sarcocystis fusiformis* und *Sarcocystis tenella. Berlin–Münchner tierärztliche Wochenschrift* **88**, 191–197 and 201–204.

Göbel, E. (1976). Elektronenmikroskopische Untersuchungen zur Feinstruktur der Zystenstadien von Pferdesarkosporidien. *Zeitschrift für Parasitenkunde* **50**, 201.

Grell, K. G. (1973). "Protozoology." Springer, Heidelberg.

Heydorn, A. O. (1977). Beiträge zum Lebenszyklus der Sarkosporidien. IX. Untersuchungen zum Zyklus von *Sarcocystis suihominis. Berlin-Münchner tierarztliche Wochenschrift* **90**, 218–224.

Heydorn, A. O. and Gestrich, R. (1976). Beiträge zum Lebenszyklus der Sarkosporidien. VII. Entwicklungsstadien von *Sarcocystis ovicanis* im Schaf. *Berlin-Münchner tierarztlische Wochenschrift* **89**, 1–5.

Heydorn, A. O. and Mehlhorn, H. (1976). Licht- und elektronenmikroskopische Untersuchungen an zwei Typen von Riesenschizonten aus dem Dünndarm des Schafes. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, I. Abteilung, Originale, Reihe* **237**, 124–137.

Heydorn, A. O. and Mehlhorn, H. (1978). Light and electron microscopic studies on *Sarcocystis suihominis.* 2. The schizogony preceding cyst formaiton. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, I. Abteilung, Originale, Reihe A* (in press).

Heydorn, A. O. and Rommel, M. (1972a). Beiträge zum Lebenszyklus der Sarkosporidien. II. Hund und Katze als Überträger der Sarkosporidien des Rindes. *Berlin-Münchner tierarztliche Wochenschrift* **85**, 121–123.

Heydorn, A. O. and Rommel, M. (1972b). Beiträge zum Lebenszyklus der Sarkosporidien. IV. Entwicklungsstadien von *S. fusiformis* in der Dünndarmschleimhaut der Katze *Berlin-Munchner tieräiztliche Wochenschrift* **85**, 333–336.

Heydorn, A. O., Ipczynski, V., Muhs, E. O. and Gestrich, R. (1974). Zystenstadien aus der Muskulatur von *Isospora hominis*-infizierten Kälbern. Bildbericht. *Berlin-Münchner tierärztliche Wochenschrift* **87**, 278.

Heydorn, A. O., Mehlhorn, H. and Gestrich, R. (1975a). Licht- und elektronenmikroskopische Untersuchungen an Cysten von *Sarcocystis fusiformis* in der Muskulatur von Kälbern nach experimenteller Infektion mit Oocysten und Sporocysten von *Isospora hominis* Railliet et Lucet, 1891. 2. Die Feinstruktur der Metrocyten und Merozoiten. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankhetien und Hygiene, I. Abteilung, Originale, Reihe A* **232**, 373–391.

Heydorn, A. O., Mehlhorn, H. and Gestrich, R. (1975b). Licht- und elektronenmikroskopische Untersuchungen an Cysten von *Sarcocystis fusiformis* in der Muskulatur von Kälbern nach experimenteller Infektion mit Oocysten und Sporocysten der grossen Form von *Isospora bigemina* des Hundes. 2. Die Feinstruktur der Cystenstadien. *Zentralblatt für Bakteriologie, Parasitenkunde,*

Infektionskrankheiten und Hygiene, I. Abteilung, Originale, Reihe A **233**, 123–137.

Heydorn, A. O., Gestrich, R., Mehlhorn, H. and Rommel, M. (1975c). Proposal for a new nomenclature of the sarcosporidia. *Zentralblatt für Baketeriologie Parasitenkunde, Infektionskrankheiten und Hygiene, I. Abteilung, Originale, Reihe A* **48**, 73–82.

Heydorn, A. O., Gestrich, R. and Janitschke, K. (1976). Beiträge zum Lebenszyklus der Sarkosporidien. VIII. Sporocysten von *Sarcocystis bovihominis* in den Faezes von Rhesusaffen (*Macaca rhesus*) und Pavianen (*Papio cynocephalus*), *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, I. Abteilung, Originale, Reihe A* **89**, 116–120.

Hewitt, J. A. (1933). Sarcocysts in man. *Journal of Pathology and Bacteriology* **36**, 133.

Janitschke, K., Protz, D. and Werner, H. (1976). Beitrag zum Entwicklungszyklus von Sarkosporidien der Grantgazelle (*Gazella granti*). *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, I. Abteilung, Originale Reihe A* **48**, 215–219.

Jeffrey, H. C. (1974). Sarcosporidiosis in man. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **68**, 17–29.

Kalyakin, V. N. and Zasukhin, D. N. (1975). Distribution of *Sarcocystis* (Protozoa, Sporozoa) in vertebrates. *Folia Parasitologica (Praha)* **22**, 289–307.

Karr, S. L. and Wong, M. M. (1975). A survey of *Sarcocystis* in nonhuman primates. *Laboratory Animal Science* **25**, 641–645.

Kartulis, S. (1893). Muskelparasiten beim Menschen. *Zeitschrift für Hygiene und Infectionskrankheiten* **13**, 2.

Kepka, O. and Scholtyseck, E. (1970). Weitere Untersuchungen der Feinstruktur von *Frenkelia* sp. (=M-Organismus). *Protistologica* **6**, 249–266.

Kim, C. S., Eugster, A. K. and Kalter, S. S. (1968). Pathologic study of the African baboon (*Papio* sp). in his native habitat. *Primates* **9**, 93–104.

Koller, L. D., Kistner, T. P. and Hudkins, G. (1977). Histopathological study of experimental *Sarcocystis* hemionilatrantris infection in fawns. *American Journal of Veterinary Research* **38**, 1205–1209.

Kutty, M. K. and Dissanaike, A. S. (1975). A case of human *Sarcocystis* infection in West Malaysia. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **52**, 503–504.

Kutty, M. K., Mohan Das, A. and Dissanaike, A. S. (1975). *Sarcocystis* infection in an orang asli: the second human case from Malaysia. *Southeast Asian Journal of Tropical Medicine and Public Health* **6**, 400–401.

Lainson, R. (1958). Observations on the development and nature of pseudocysts and cysts of *Toxoplasma gondii. Transactions of the Royal Society of Tropical Medicine and Hygiene* **52**, 396–407.

Lainson, R. and Shaw, J. J. (1971). *Sarcocystis gracilis* n. sp. from the Brazilian tortoise *Kinosternon scorpioides. Journal of Protozoology* **18**, 365–372.

Lankester, E. R. (1882). On *Drepanidium ranarum* the cell-parasite of the frog's blood and spleen. *Quarterly Journal of Microscopical Science* **12**, 53–65.

Levine, N. D. (1973). "Protozoan parasites of domestic animals and of man," 2nd edition. Burgess, Minneapolis.

Levine, N. D. (1977). Nomenclature of *Sarcocystis* in the ox and sheep and of fecal coccidia of the dog and cat. *Journal of Parasitology* **63**, 36–51.

Lindemann, C. (1863). Psorospermes chez l'homme. *Bulletin de la Société Impériale des Naturalistes de Moscow* **36**, 425–436.

Lorenz, H. (1904). "Muskelerkrankungen." Wein, Heidelberg.

Ludvík, J. (1960). The electron microscopy of *Sarcocystis miescheriana* (Kühn, 1865). *Journal of Protozoology* **7**, 128–135.

Machulskii, S. N. and Miskaryan, N. D. (1958). Description of sarcosporidian species. [In Russian]. *Transactions of the Buryat-Mongolian Zooveterinarian Institute* **13**, 297–299.

Mahrt, J. L. (1973). *Sarcocystis* in dogs and its probable transmission from cattle. *Journal of Parasitology* **59**, 588–589.

Mandour, A. M. (1964). A report of the presence of *Sarcocystis* in different hosts. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **58**, 287–288.

Mandour, A. M. (1969). *Sarcocystis nesbitti* n. sp. from the rhesus monkey. *Journal of Protozoology* **16**, 353–354.

Markus, M. B., Killick-Kendrick, R. and Garnham, P. C. C. (1974). The coccidial nature and life-cycle of *Sarcocystis*. *Journal of Tropical Medicine and Hygiene* **77**, 248–259.

McConnell, E. E., Basson, P. A., De Vos, V., Myers, B. J. and Kuntz, R. E. (1974). A survey of diseases among 100 free-ranging baboons (*Papio ursinus*) from the Krüger National Park. *Onderstepoort Journal of Veterinary Research* **41**, 97–168.

Mehlhorn, H. (1972a). Elektronenmikroskopische Untersuchungen an Entwicklungsstadien von *Eimeria maxima*. 1. Die Feinstruktur der Makrogameten. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, I. Abteilung, Originale, Reihe A* **39**, 161–182.

Mehlhorn, H. (1972b). Elektronenmikroskopische Untersuchungen an Entwicklungsstadien von *Eimeria maxima*. 3. Der Differenzierungsprozeß der Mikrogameten unter besonderer Berücksichtigung der Kernteilungen. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, I. Abteilung, Originale, Reihe A* **40**, 243–260.

Mehlhorn, H. (1975). Elektronenmikroskopischer Nachweis von alkalischer Phosphatase und ATP-ase in Cystenstadien von *Sarcocystis tenella* aus der Schlundmuskulatur von Schafen. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, I. Abteilung, Originale, Reihe A* **46**, 95–109.

Mehlhorn, H. and Heydorn, A. O. (1976). Licht- und elektronenmikroskopische Untersuchungen an zwei Typen von Riesenschizonten (sog. Globidien) aus dem Labmagen des Schafes. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, I. Abteilung, Originale, Reihe A* **236**, 386–406.

Mehlhorn, H. and Heydorn, A. O. (1977). Licht- und elektronenmikroskopische Untersuchungen an *Sarcocystis suihominis*. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, I. Abteilung, Originale, Reihe A* **239**, 124–139.

Mehlhorn, H. and Markus, M. B. (1976). Electron microscopy of stages of *Isospora felis* of the cat in the mesenteric lymph nodes of the mouse. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, I. Abteilung, Originale, Reihe A* **51**, 15–24.

Mehlhorn, H. and Scholtyseck, E. (1973). Elektronenmikroskopische Untersuchungen an Cystenstadien von *Sarcocystis tenella* aus der Oesophagus Muskulatur des Schafes. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, I. Abteilung, Originale, Reihe A* **41**, 291–310.

Mehlhorn, H. and Scholtyseck, E. (1974). Licht- und elektronenmikroskopische Untersuchungen an Entwicklungsstadien von *Sarcocystis tenella* aus der Darmwand der Hauskatze. 1. Die Oocysten und Sporocysten. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, I. Abteilung, Originale, Reihe A* **43**, 251–270.

Mehlhorn, H. and Sénaud, J. (1975). Action lytique des bactéries présentes dans les kystes de *Sarcocystis tenella*. *Archives of Microbiology* **104**, 241–244.

Mehlhorn, H., Sénaud, J. and Scholtyseck, E. (1974). Études ultrastructurales de *Toxoplasma gondii, Sarcocystis tenella, Besnoitia jellisoni* et *Frenkelia* sp. Distribution de phosphatase acide et de polysaccharides chez parasite et l'hôte. *Protistologica* **10**, 21–43.

Mehlhorn, H., Heydorn, A. O. and Gestrich, R. (1975a). Licht- und elektronenmikroskopische Untersuchungen an Cysten von *Sarcocystis fusiformis* in de Muskulatur von Kälbern nach experimenteller Infektion mit Oocysten und Sporocysten von *Isospora hominis*. 1. Zur Entstehung der Cyste und der Cystenwand. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, I. Abteilung, Originale, Reihe A* **231**, 301–322.

Mehlhorn, H., Heydorn, A. O. and Gestrich, R. (1975b). Licht- und elektronenmikroskopische Untersuchungen an Cysten von *Sarcocystis fusiformis* in der Muskulatur von Kälbern nach experimenteller Infektion mit Oocysten und Sporocysten der grossen Form von *I. bigemina* des Hundes. 1. Zur Entstehung der Cyste und der Cystenwand. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, I. Abteilung, Originale, Reihe A* **232**, 392–409.

Mehlhorn, H., Heydorn, A. O. and Gestrich, R. (1975c). Licht- und elektronenmikroskopische Untersuchungen an Cysten von *Sarcocystis ovicanis*. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, I. Abteilung, Originale, Reihe A* **48**, 83–93.

Mehlhorn, H., Sénaud, J., Heydorn, A. O. and Gestrich, R. (1975d). Comparaison des ultrastructures des kystes de *Sarcocystis fusiformis* Railliet, 1897 dans la musculature du boeuf, après infection expérimentale par des sporokystes d'*Isospora hominis* et par des grandes formes d'*Isospora bigemina* du chien et du chat. *Protistologica* **11**, 445–455.

Mehlhorn, H., Weber, G., Schein, E. and Büscher, G. (1975e). Elektronenmikroskopische Untersuchung an Entwicklungstadien von *Theileria annulata* im Darm und in der Hämolymphe von *Hyalomma anatolicum excavatum*. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, I. Abteilung, Originale, Reihe A* **48**, 137–150.

Mehlhorn, H., Hartley, W. T. and Heydorn, A. O. (1976). Comparative electron microscopical study on the cyst wall in thirteen *Sarcocystis* species. *Protistologica* **12**, 451–467.

Mehlhorn, H., Heydorn, A. O. and Janitschke, K. (1977). Light and electron microscopical study on sarcocysts from muscles of the rhesus monkey (*Macaca mulatta*), baboon (*Papio cynocephalus*) and tamarin (*Saguinus* (= *Oedipomidas*) *oedipus*). *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, I. Abteilung, Originale, Reihe A* **51**, 165–178.

Mehlhorn, H. and Heydorn, A. O. (1978). Light and electron microscopic studies on *Sarcocystis suihominis*. III. The gamogony in human tissue cells. *Zeitschrift für Parasitenkunde* (in press).

Miescher, F. (1843). Über eigenthümliche Schläuche in den Muskeln einer Hausmaus. *Berichte der Verhand Lungen der naturforschenden Geselschaft in Basel* **5**, 198–202.

Munday, B. L. and Corbould, A. (1974). The possible role of the dog in the epidemiology of ovine sarcosporidiosis. *British Veterinary Journal* **130**, ix.

Munday, B. L. and Rickard, M. D. (1974). Is *Sarcocystis tenella* two species? *Australian Veterinary Journal* **50**, 558–559.

Munday, B. L., Barker, I. K. and Rickard, M. D. (1975). The developmental cycle of a species of *Sarcocystis* occurring in dogs and sheep, with observations on pathogenicity in the intermediate host. *Zeitschrift für Parasitenkunde* **46**, 111–123.

Nelson, B., Cosgrove, G. E. and Gengozian, N. (1966). Diseases of an imported primate *Tamarinus nigricollis*. *Laboratory Animal Science* **16**, 255–275.

Overdulve, J. P. (1970). The identity of *Toxoplasma gondii* with *Isospora*. *Proceedings: koninklijke Nederlandse Akademie van Wettenschappen* **73**, 130–151.

Pacheco, N. D. and Fayer, R. (1977). Fine structure of *Sarcocystis cruzi* schizonts. *Journal of Protozoology* **24**, 382–388.

Pellérdy, L. P. (1974). Coccidia and Coccidiosis, (2nd edition). Parey, Berlin.

Pfeiffer, L. (1890). Sarcotoxin. *Archiv für Pathologie, Anatomie, Physiologie und Klinische Medizin* **122**, 552–573.

Piekarski, G. und Witte, H. M. (1970). Der Erreger der Toxoplasmose—ein spezifischer Katzenparasit. *Umschau* **11**, 342–343.

Porchet-Henneré, E. (1975). Quelques précisions sur l'ultrastructure de *Sarcocystis tenella*. 1. L'endozoite (après coloration négative). *Journal of Protozoology* **22**, 214–220.

Powell, E. C. and McCarley, J. B. (1975). A murine *Sarcocystis* that causes an *Isospora*-like infection in cats. *Journal of Parasitology* **61**, 928–931.

Ratzel, F. (1868). Beschreibung einiger neuer Parasiten. 4. Psorospermien in Affenmuskeln. *Archiv für Naturgeschichte Berlin* **34**, 150–156.

Rivolta, S. (1878). Della gregarinosi dei polli e dell 'ordinamento delle gregarine e dei psorospermi degli animali domestici. *Lombard Giornale del Anatomia e Fisiologia e Patologia dei Animales* **10**, 220–260.

Rommel, M. and Geisel, O. (1975). Untersuchungen über die Verbreitung und den Lebenszyklus einer Sarkosporidienart des Pferdes (*Sarcocystis equicanis*). *Berlin-Münchner tierärztliche Wochenschrift* **88**, 468–471.

Rommel, M. and Heydorn, A. O. (1972). Beiträge zum Lebenszyklus der Sarkosporidien. III. *Isospora hominis* (Railliet et Lucet, 1891) Wenyon, 1923, eine Dauerform der Sarkosporidien des Rindes und des Schweins. *Berlin-Münchner tierärztliche Wochenschrift* **85**, 143–145.

Rommel, M., Heydorn, A. O. and Gruber, F. (1972). Beiträge zum Lebenszyklus der Sarkosporidien, I. Die Sporocyste von *S. tenella* in den Fäzes der Katze. *Berlin-Münchner tierärztliche Wochenschrift* **85**, 101–105.

Rommel, M. and Krampitz, H. E. (1975). Beiträge zum Lebenszyklus der Frenkelien. I. Die Identität von *Isospora buteonis* aus dem Mäusebussard mit einer Frenkelienart (*F. clethrionomyobuteonis*) aus der Rötelmaus. *Berlin-Münchner tierärztliche Wochenschrift* **88**, 338–340.

Rommel, M., Heydorn, A. O., Fischle, B. and Gestrich, R. (1974). Beiträge zum Lebenszyklus der Sarkosporidien. V. Weitere Endwirte der Sarkosporidien von Rind, Schaf und Schwein und die Bedeutung des Zwischenwirts für die Verbreitung dieser Parasitose. *Berlin-Münchner tierärztliche Wochenschrift* **87**, 392–396.

Ruiz, A. and Frenkel, J. K. (1976). Recognition of cyclic transmission of *Sarcocystis muris* by cats. *Journal of Infectious Diseases* **133**, 409–418.

Rzepczyk, C. M. (1974). Evidence of a rat-snake life cycle for *Sarcocystis*. *International Journal for Parasitology* **4**, 447–449.

Rzepczyk, C. M. and Scholtyseck, E. (1976). Light and electron microscope studies on the *Sarcocystis* of *Rattus fuscipes*, an Australian rat. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, I. Abteilung, Originale, Reihe* **50**, 137–150.

Schein, E. (1975). Zum Entwicklungszyklus von *Theileria annulata* (Dschunkowsky und Luhs, 1904) in der Uberträgerzecke *Hyalomma anatolicum excavatum* (Koch, 1844). *Habil-Schrift*, Freien Universität, Berlin.

Scholtyseck, E., Mehlhorn, H. and Hammond, D. M. (1971). Fine structure of macrogametes and oocysts of coccidia and related organisms. *Zeitschrift für Parasitenkunde* **37**, 1–43

Scholtyseck, E., Mehlhorn, H. and Hammond, D. M. (1972). Electron microscope studies of microgametogenesis in coccidia and related groups. *Zeitschrift für Parasitenkunde* **38**, 95–131.

Scholtyseck, E., Mehlhorn, H. and Müller, B. E. G. (1974). Feinstruktur der Cyste und der Cystenwand von *Sarcocystis tenella, Besnoitia jellisoni, Frenkelia* sp. und *Toxoplasma gondii. Journal of Protozoology* **21**, 284–294.

Scholtyseck, E. and Piekarski, G. (1965). Elektronenmikroskopische Untersuchungen an Merozoiten von Eimerien (*E. perforans* u. *E. stiedae*) und *Toxoplasma gondii.* Zur systematischen Stellung von *T. gondii. Zeitschrift für Parasitenkunde* **26**, 91–115.

Sénaud, J. (1967). Contribution à l'étude des sarcosporidies et toxoplasmes. *Protistologica* **3**, 167–232.

Sénaud, J. (1969). Ultrastructure des formations kystiques de *Besnoitia jellisoni* (Frenkel, 1953) Protozoaire, Toxoplasmea, parasite de la souris. *Protistologica* **5**, 413–430.

Sénaud, J. and Mehlhorn, H. (1975). Étude ultrastructurale des coccidies formant des kystes: *Toxoplasma gondii, Sarcocystis tenella, Besnoitia jellisoni* et *Frenkelia* sp. (Sporozoa). II. Mise en évidence de l'ADN et de l'ARN au niveau des ultrastructures. *Annales de la station Biologique de Besse en Chandesse* **9**, 111–156.

Sénaud, J., Vedrely, R. and Tronche, P. (1968). Sur la nature de la substance toxique des kystes de Sarcosporidies du mouton (Toxoplasmea), active sur le lapin. *Compte Rendu de l'Academie des Sciences, Paris* **266**, 1137–1138.

Sheffield, H. G. (1968). Observations on the fine structure of the "cyst stage" of *Besnoitia jellisoni. Journal of Protozoology* **15**, 685–693.

Sheffield, H. G. and Melton, M. L. (1970). *Toxoplasma gondii:* the oocyst, sporozoite and infection of cultured cells. *Science* **167**, 892–893.

Sheffield, H. G., Frenkel, J. K. and Ruiz, A. (1976). Ultrastructure of *Sarcocystis muris* cysts. *Program and Abstracts of 51st annual meeting of the American Society of Parasitologists, San Antonio,* 54–55.

Simpson, C. F. and Forrester, D. J. (1973). Electron microscopy of *Sarcocystis* sp.: cyst wall, micropore, rhoptries, and an unidentified body. *International Journal for Parasitology* **3**, 467–470.

Tadros, W. and Laarman, J. J. (1975). The weasel, *Mustela nivalis*, as the final host of a *Sarcocystis* of the common European vole, *Microtus arvalis. Proceedings: Koninklijke Nederlandse Akademie van Wetenschappen Ser. B* **78**, 325–326.

Tadros, W. and Laarman, J. J. (1977). The cat *Felis catus* as the final host of *Sarcocystis cuniculi* BRUMPT, 1913 of the rabbit *Oryctolagus cuniculus. Proceedings: Koninklijke Nederlandse Akademie van Wetenschappen* **86**, 351–253.

Vershinin, I. I. (1973). O tsikle razvitiya *Sarcocystis tenella. Veterinariya* **10**, 75–78.

Vetterling, J. M., Pacheco, N. D. and Fayer, R. (1973). Fine structure of gametogony and oocyst formation in *Sarcocystis* sp. in cell culture. *Journal of Protozoology* **20**, 613–621.

Viles, J. M. and Powell, E. C. (1976). The ultrastructure of the cyst wall of a murine *Sarcocystis. Zeitschrift für Parasitenkunde* **49**, 127–132.

Wallace, G. D. and Frenkel, J. K. (1975). *Besnoitia* sp.: recognition of cyclic transmission by cats. *Science* **188**, 369–371.

Work, K. and Hutchison, W. M. (1969). The new cyst of *Toxoplasma gondii*. *Acta Pathologia und Microbiologica Scandinavica* **77**, 414–424.

Zaman, V. (1970). *Sarcocystis* sp. in the slow loris *Nycticebus coucang*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **64**, 195–196.

Zaman, V. and Colley, F. C. (1975). Light and electron microscopic observations of the life cycle of *Sarcocystis orientalis* sp. n. in the rat (*Rattus norvegicus*) and the Malaysian reticulated python (*Python reticulatus*). *Zeitschrift für Parasitenkunde* **47**, 169–185.

Zeve, V. H., Price, D. L. and Herman, C. M. (1966). Electron microscope studies of *Sarcocystis* sp. *Experimental Parasitology* **18**, 338–346.

Abbreviations for Figures

A	Polysaccharide granules
AS	Amorphous material
B	Vesicle-like invaginations of the primary cyst wall (PH)
BP	Bulbous ends of the wave-like protrusions of the PH
C	Conoid
CA	Chamber-like hollow in the ground substance of the cyst
CH	Chromosomes
CR	Crystalloidal structure
CW	Cyst wall as seen in the light microscope or scanning electron microscope
DI	Dense inclusion within the ground substance
DG	Dense granules
DV	Double-walled vesicle
EN	Daughter cell formation by endodyogeny
ER	Endoplasmic reticulum
F	Fibrillar elements
FG	Fine granular structures with regular spacing
GO	Golgi apparatus
GS	Ground substance of the cyst
HC	Host cell
HM	Outer limiting membrane of the host cell
I	Dense inclusion within mitochondrion
IM	Inner membrane of the pellicle
IN	Invagination
L	Lipid
LN	Limiting membrane of the neighbouring cell
LP	Limiting membrane of the parasitophorous vacuole
LV	Large, square protrusion of the primary wall
MB	Bundle of microtubules
MC	Metrocyte
ME	Merozoite
MFI	Muscle fibrils
MI	Mitochondrion
MIH	Mitochondrion of the host cell
MIN	Mitochondrion of the neighbouring cell
MM	Middle membrane of the pellicle
MN	Micronemes
MNA	Microneme-like structure

MP	Micropore
MT	Microtubules
N	Nucleus
NC	Neighbouring cell
NH	Nucleus of the host cell
NP	Nuclear pore
NU	Nucleolus
O	Opening in the conoid
OM	Outer membrane of the pellicle
OS	Osmiophilic structure
OW	Oocyst wall
P	Polar ring
PA	Palisade-like protrusion of the PH
PE	Pellicle
PH	Primary cyst wall
PP	Posterior polar ring
PV	Parasitophorous vacuole
R	Rhoptries
RB	Ribosomes
RH	Remains of the host cell
RI	Conoidal rings
RS	Residual body
S	Sporozoite
SE	Septum
SH	Secondary cyst wall
SL	Spindle-like structure
SP	Sporocyst
ST	Subpellicular microtubules
SW	Sporocyst wall
UL	Underlying osmiophilic material
UM	Unit membrane
V	Vacuole
VE	Vesicles
VL	Vesicle-like protrusion of the PH
VW	Finger-like protrusion of the PH
WF	Wall-forming bodies within macrogametes
WP	Wave-like protrusion of the PH
Z	Z-line of the sarcomeres of the muscle
ZA	Cauliflower-like protrusion of the PH

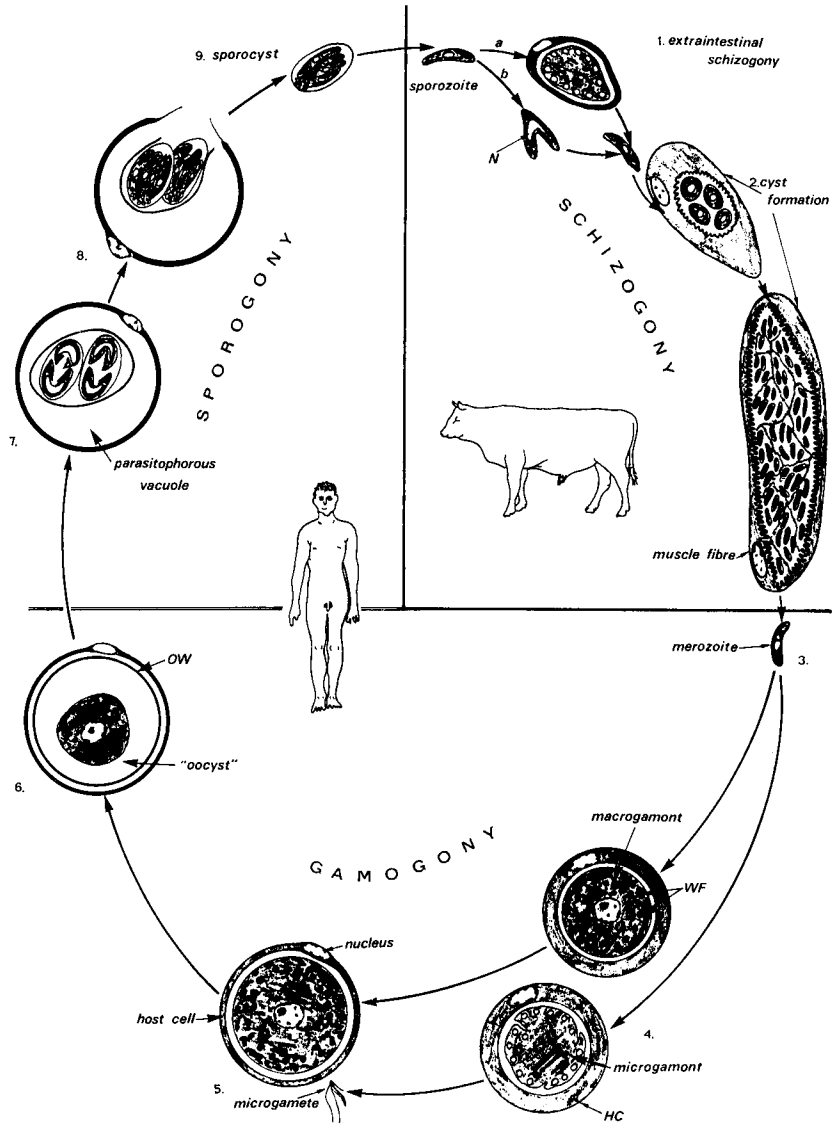


FIG. 1. Life cycle of *Sarcocystis bovihominis* parasitizing man ("predator") and cattle ("prey"). When ingested by the prey, the sporozoites from sporocysts initiate an extraintestinal schizogony (1), where numerous schizonts (1a) or endodyogeny-stages (1b) are formed, mostly within endothelial cells. About one month later merozoites resulting from schizogony or endodyogeny give rise to cysts within muscle fibres. These cysts finally contain (about three months p.i.) predominantly merozoites, thus being ready for transmission when the muscles are ingested by the predator. The merozoites (3) grow directly to sexual stages (4, gamonts), having penetrated the intestinal cells of the predator. The parasites are always situated within a clear parasitophorous vacuole in the host cell. After fertilization (5) the zygote (erroneously termed "oocyst") is surrounded by a wall. Oocysts sporulate in situ in the subepithelial tissue (7) and are released into the lumen of the gut; stages in the faeces are mainly free sporocysts (9) which have broken out of the fragile oocysts.

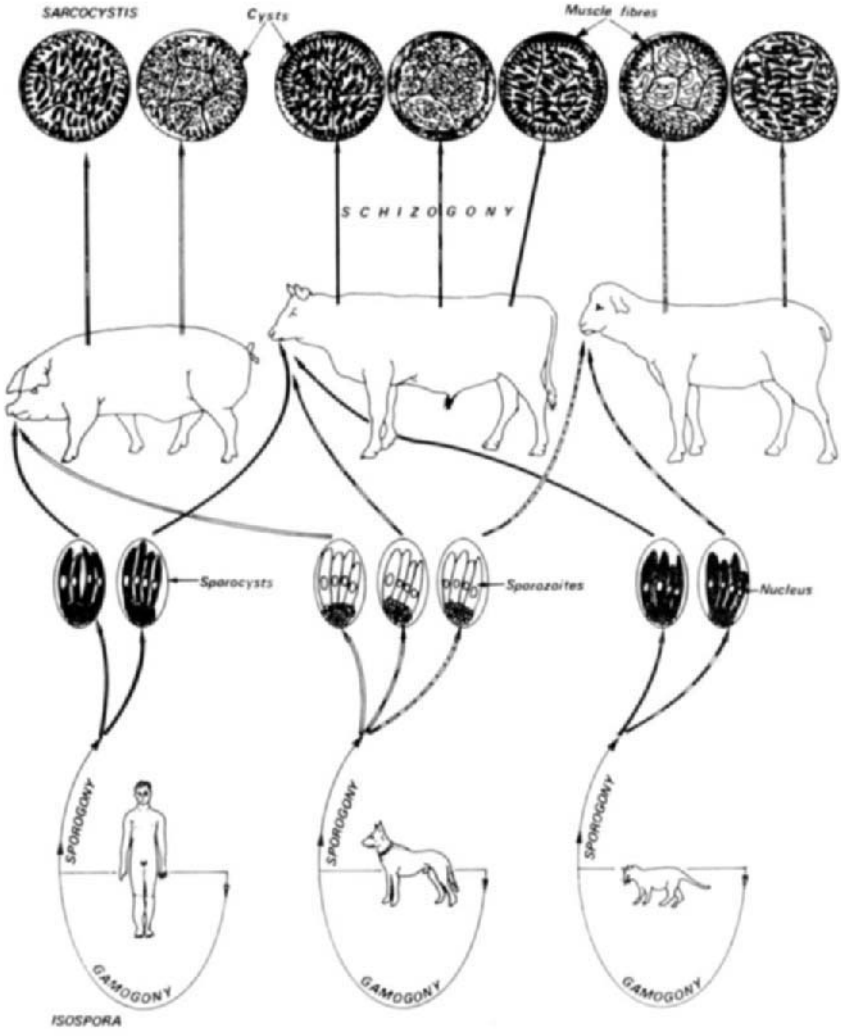
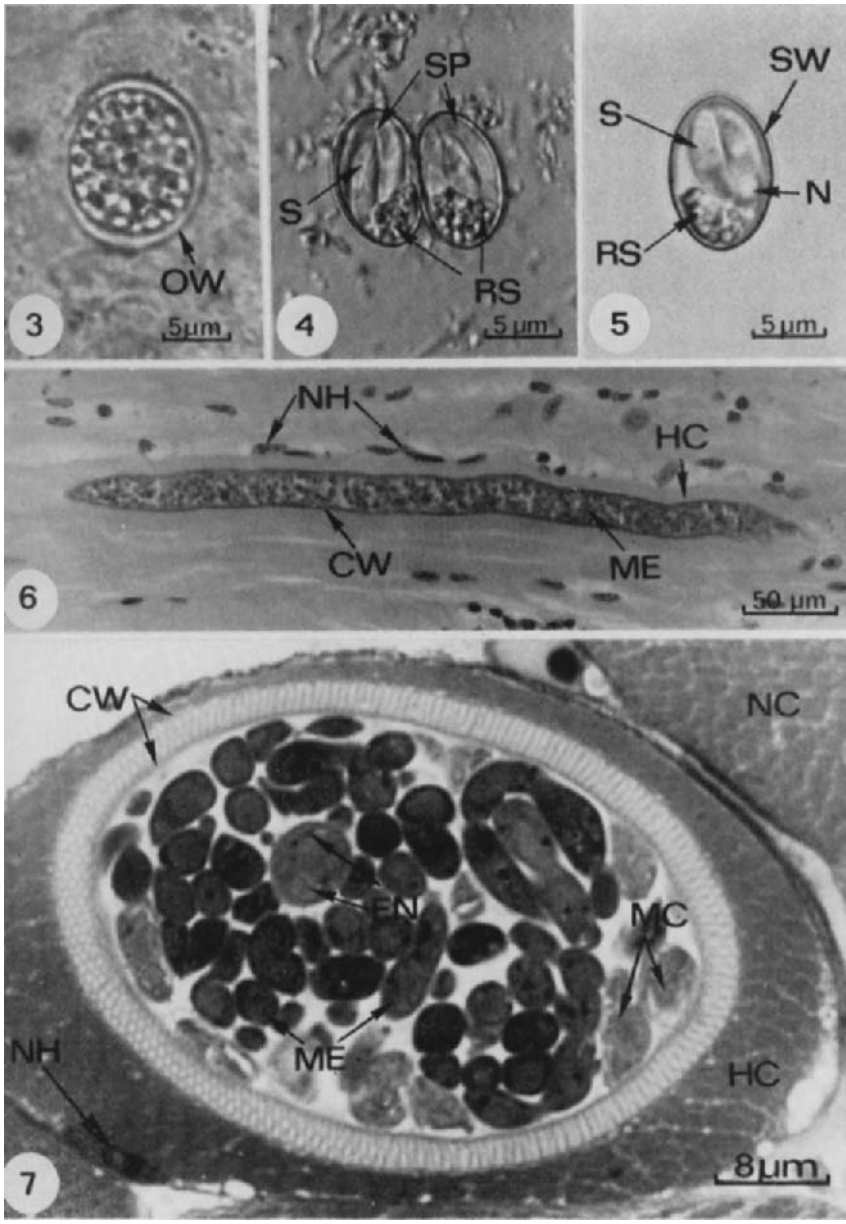


FIG. 2. Diagrammatic representation of the life cycles of seven *Sarcocystis* species, the stages of which were known in the three final hosts as *Isospora* species. *Sarcocystis miescheriana* of swine is replaced by two species, *S. fusiformis* by three species and *S. tenella* of sheep by two species (compare Table 1).



FIGS 3–7. Light micrographs.
FIG. 3. *S. tenella*. Unsporulated oocyst within the intestine of a cat.
FIG. 4. *S. bovicanis*. Sporulated oocyst from fresh faeces of a dog.
FIG. 5. *S. bovihominis*. Free sporocyst from human faeces.
FIG. 6. *S. bovicanis*. Longitudinal section through a thin-walled, mature cyst.
FIG. 7. *S. ovicanis* (microscopic form of *S. "tenella"*). Cross section through a mature cyst, which has a cyst wall with numerous protrusions. (Semi-thin section.)

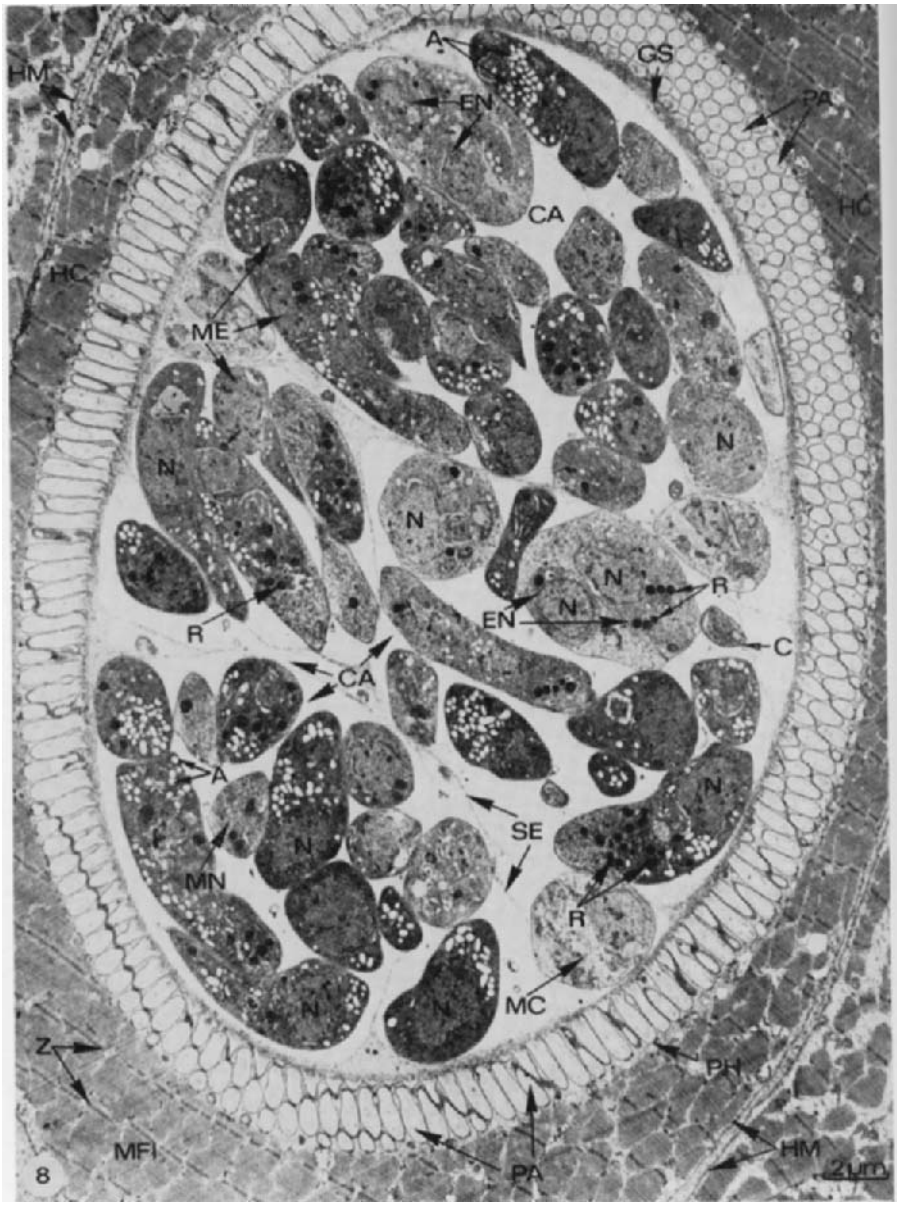


FIG. 8. Electron micrograph of *Sarcocystis ovicanis* from sheep. Cross section through a mature cyst.

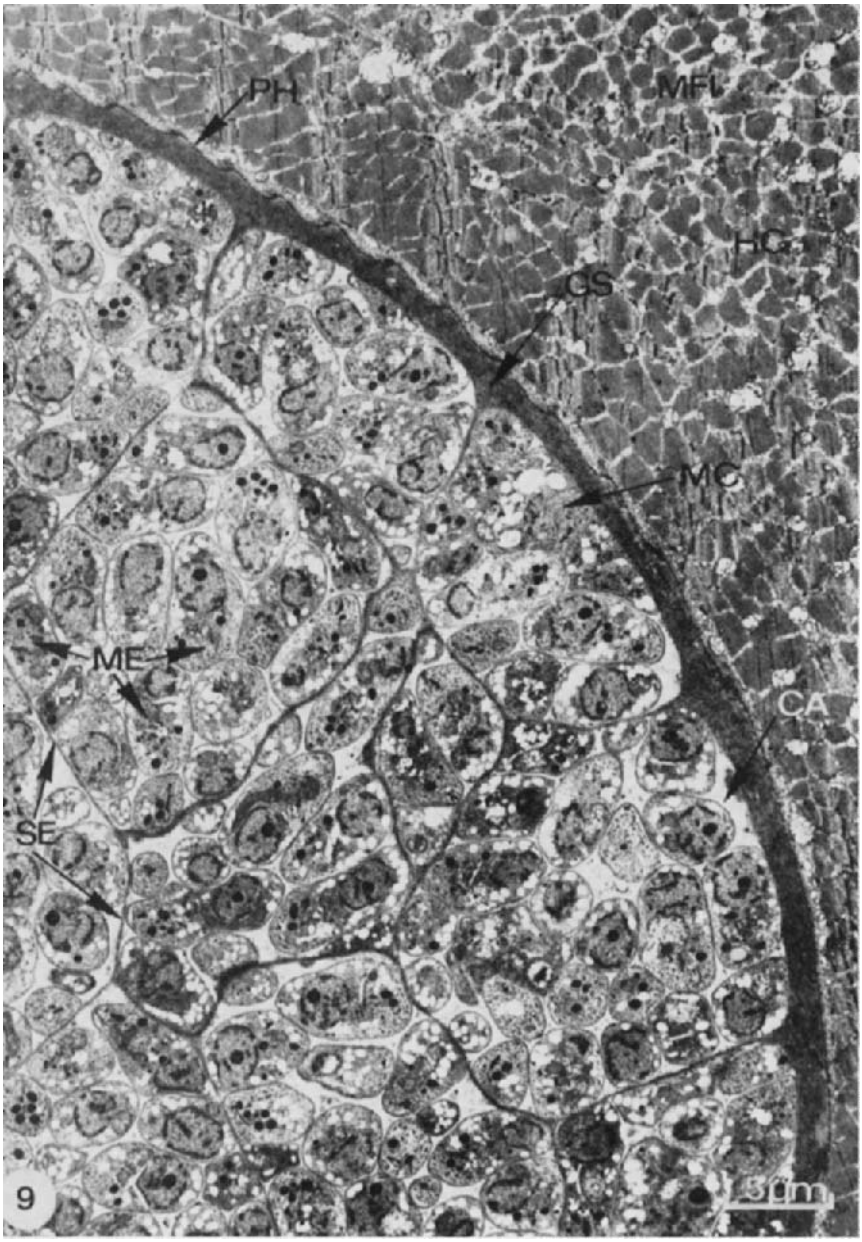


FIG. 9. Electron micrograph of *Sarcocystis* sp. from rhesus monkey. Cross section through the periphery of a mature cyst.

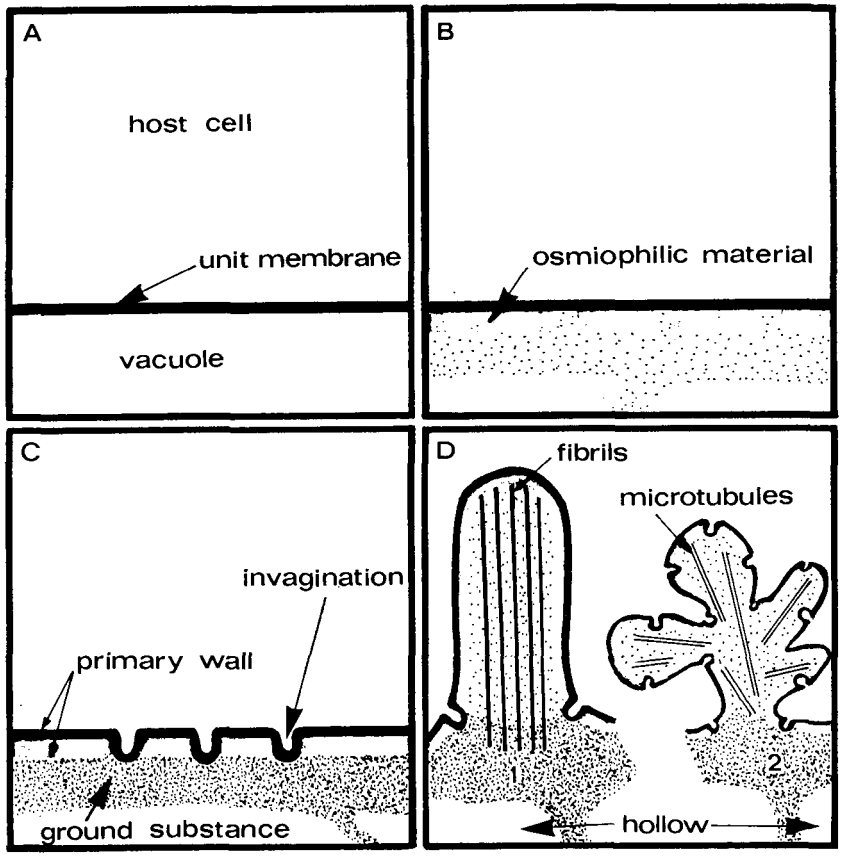


FIG. 10. Diagrammatic representation of the development of the primary cyst wall and the underlying ground substance of the cyst. In mature cysts the primary cyst wall may form protrusions (D 1 = *S. bovihominis*; D 2 = *S. tenella*) or not.

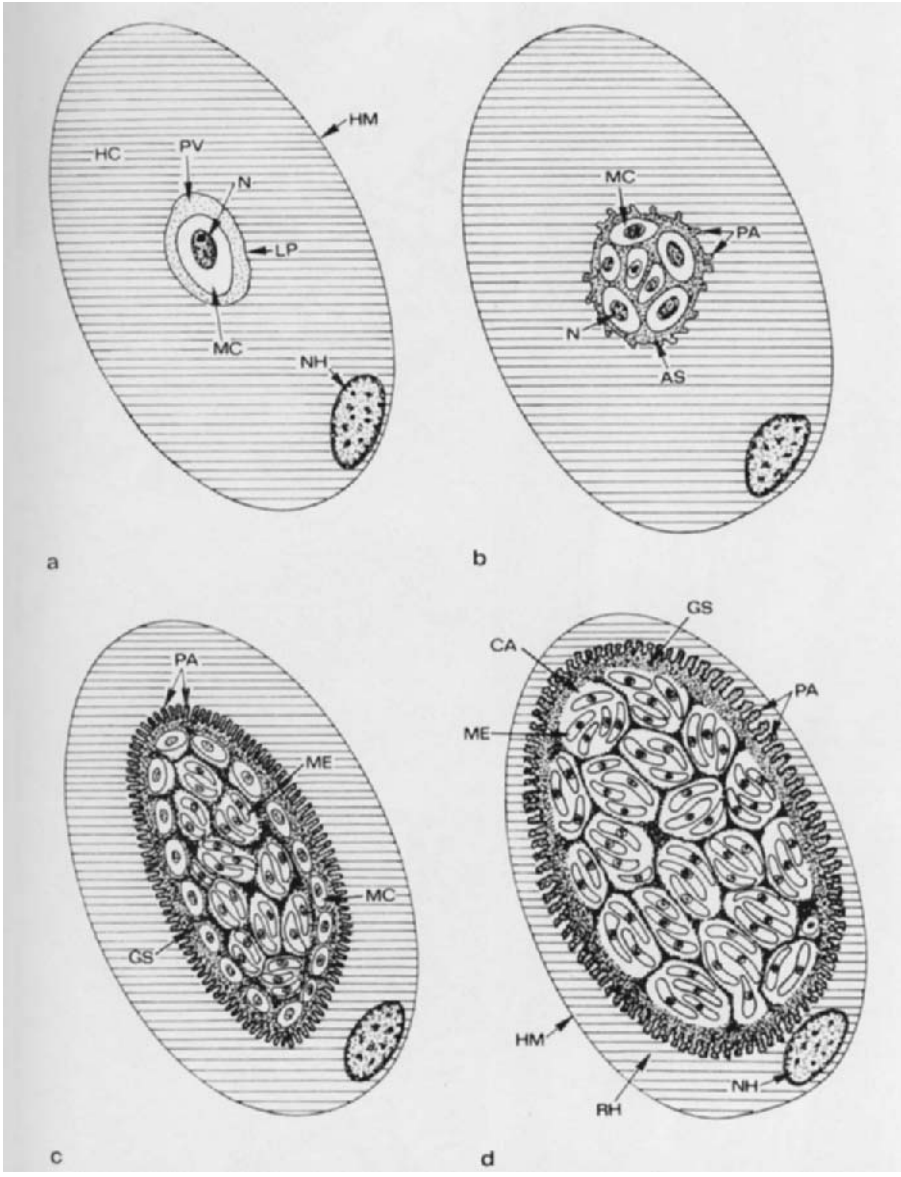
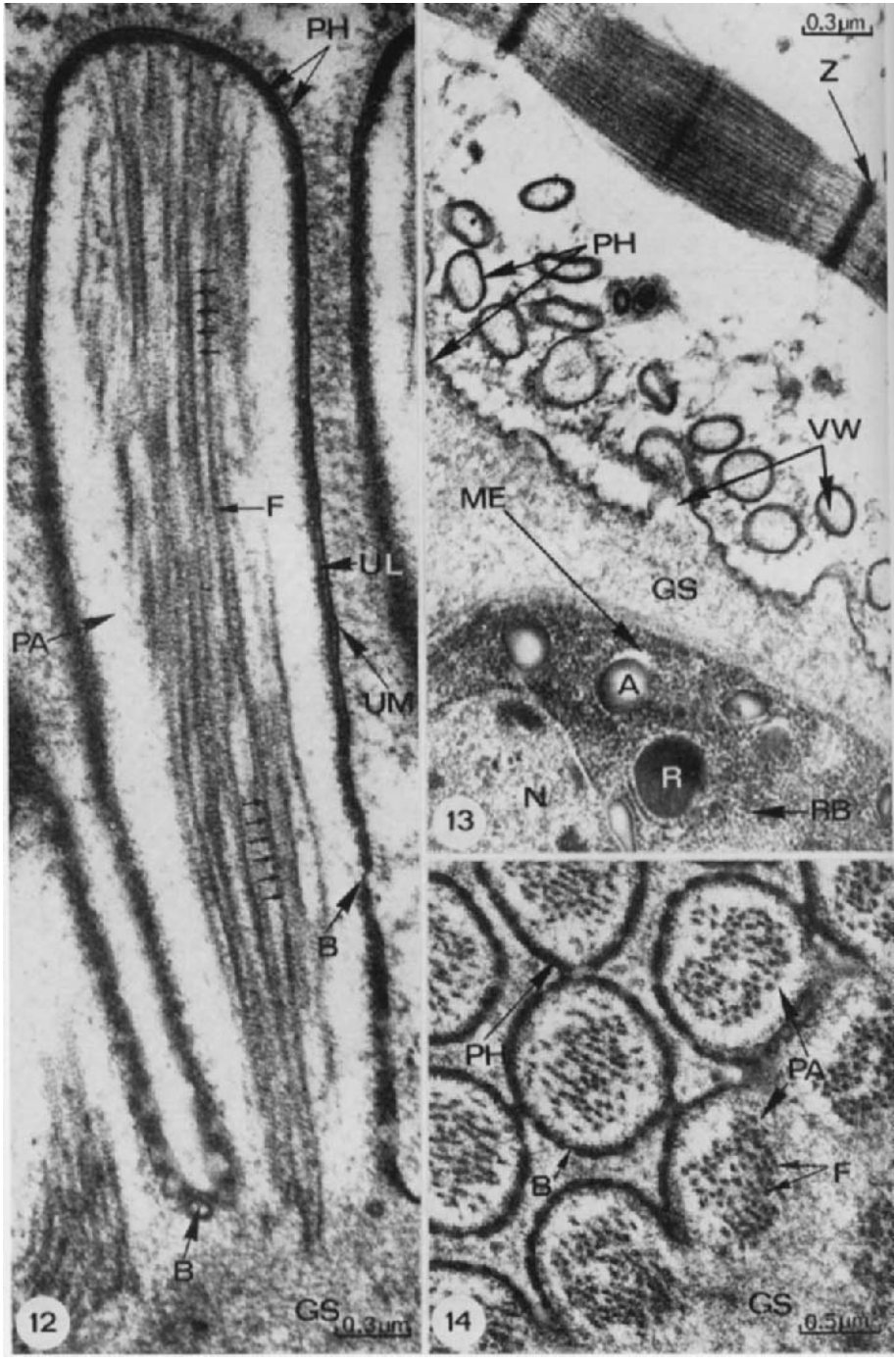


FIG. 11. Diagrammatic representation of the development of a sarcosporidian cyst within a muscle fibre. Apart from the variations in the peculiar protrusions of the primary cyst wall this scheme is characteristic of all *Sarcocystis* species. The development starts about one month p.i. (a) with the formation of a parasitophorous vacuole and is finished two months later, when the chamber-like hollows of the ground substance (CA, GS) contain infectious merozoites (d).



FIGS 12–14. Electron micrographs of sarcocysts from bovine muscle.

FIG. 12. *S. bovihominis*. Longitudinal section through a palisade-like protrusion of the primary cyst wall containing numerous fibrillar elements. Note the regular striation of the fibrillar structures (small arrows).

FIG. 13. *S. bovicanis*. Section through the periphery of a mature cyst. Note that only a few protrusions (VW) are present following a course along the surface of the cyst.

FIG. 14. *S. bovihominis*. Cross section through the palisade-like protrusions of a mature cyst.

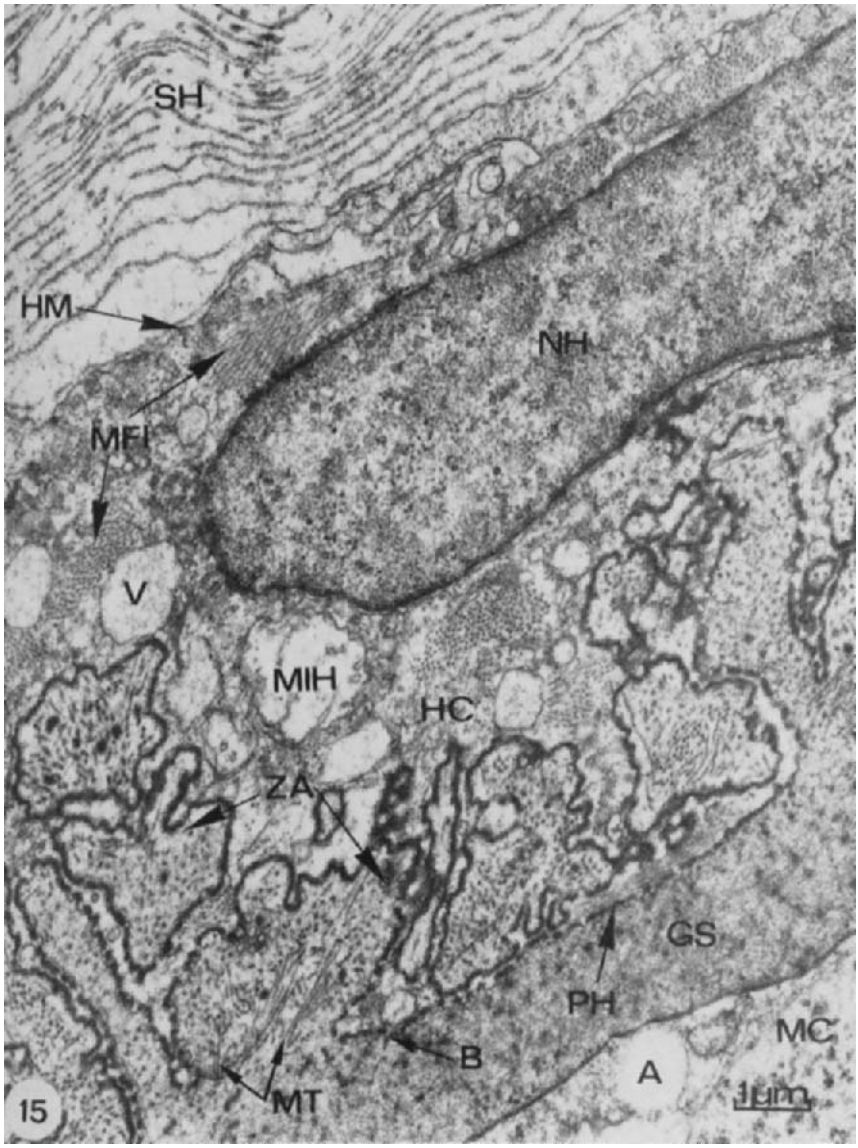


FIG. 15. Electron micrograph of *Sarcocystis tenella* from sheep. Cross section through the periphery of a mature cyst with numerous cauliflower-like protrusions.

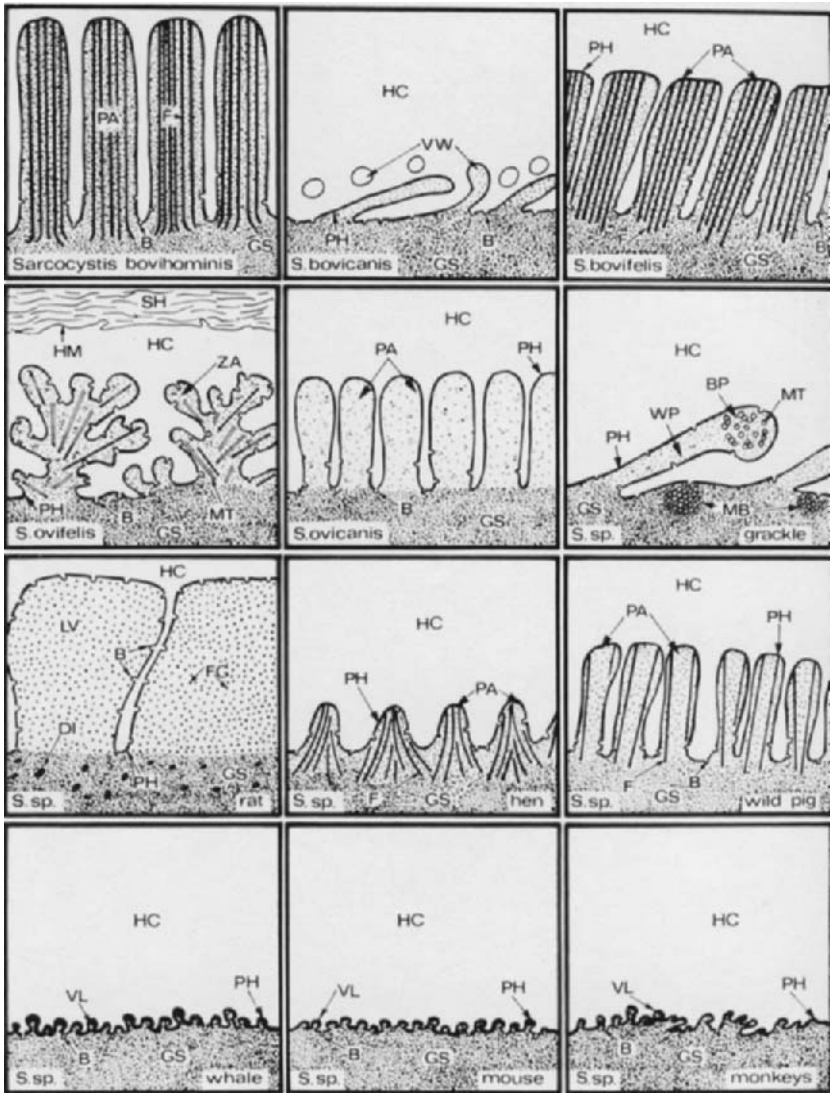
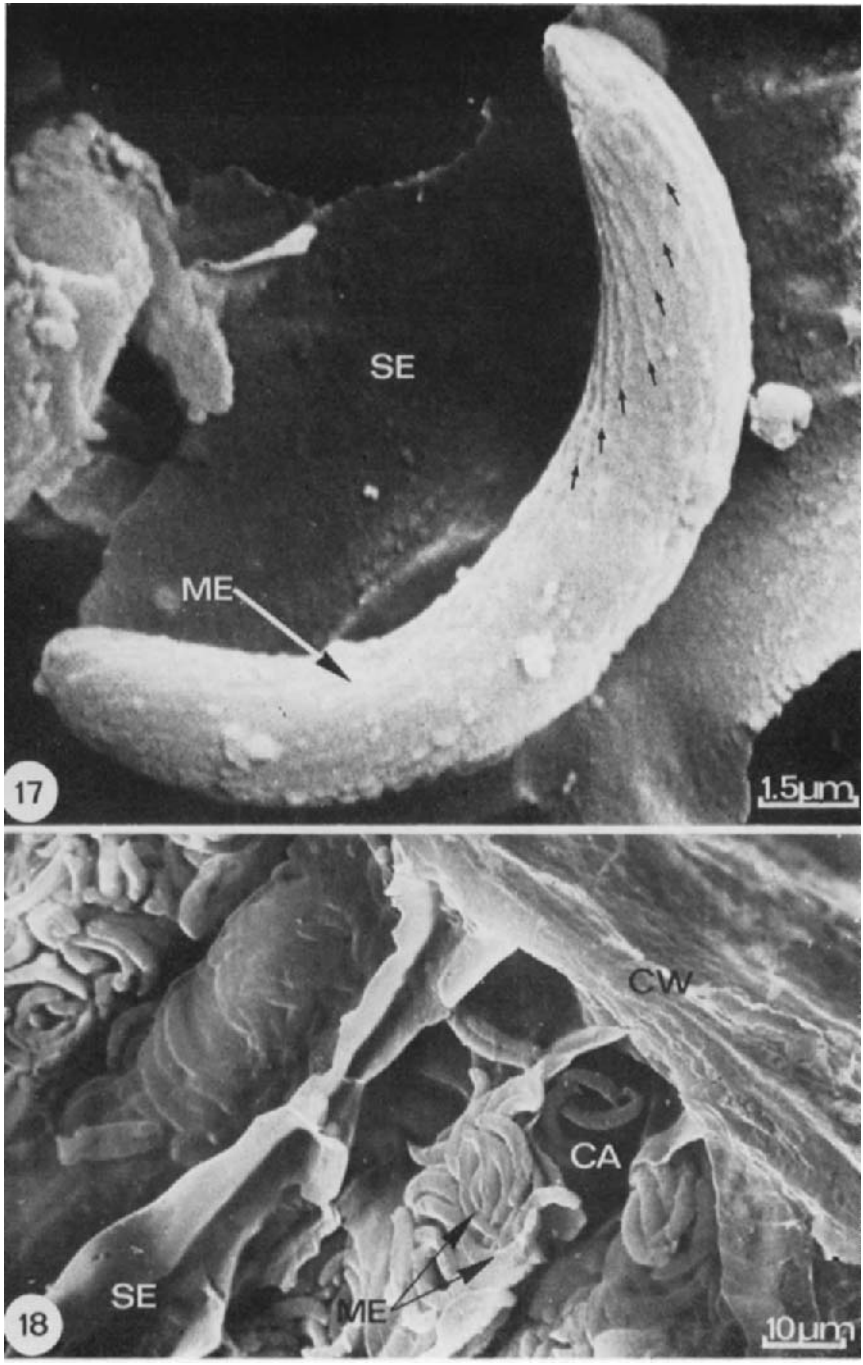


FIG. 16. Diagrammatic representation of the differentiations of the primary cyst wall in some *Sarcocystis* species. Rat = *Rattus lutreolus* from Australia; whale = *Physeter catadon* from Australia (joint investigations with Dr. Hartley); monkeys = rhesus monkey, baboon and tamarin (cf. Mehlhorn *et al.*, 1977).



FIGS 17–18. Scanning electron micrographs of *Sarcocystis tenella* from sheep.
FIG. 17. Surface view of entire merozoite. Note the rib-like structures (small arrows).
FIG. 18. Section through the periphery of a cyst showing the chamber-like hollows (CA) with numerous merozoite (ME).

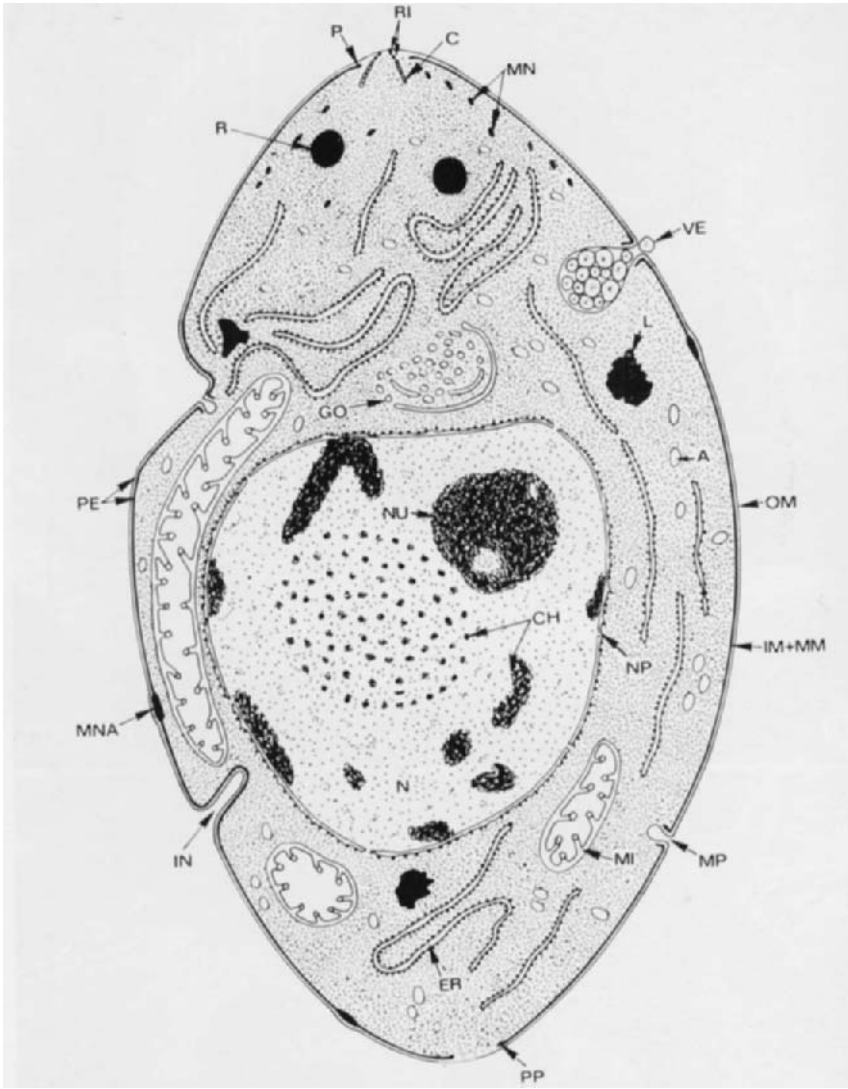


FIG. 19. Diagrammatic representation of a metrocyte (e.g. *S. bovicanis*).

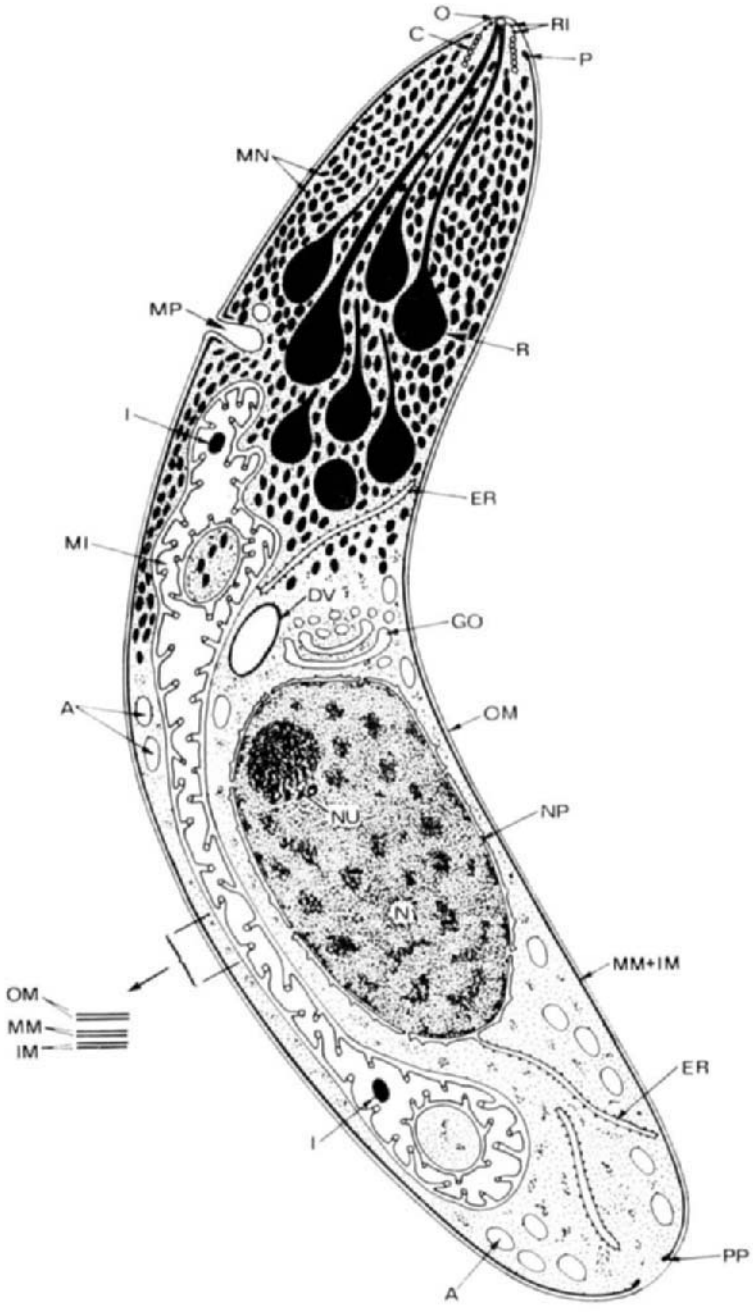


FIG. 20. Diagrammatic representation of a merozoite from a cyst (e.g. *S. tenella*.)

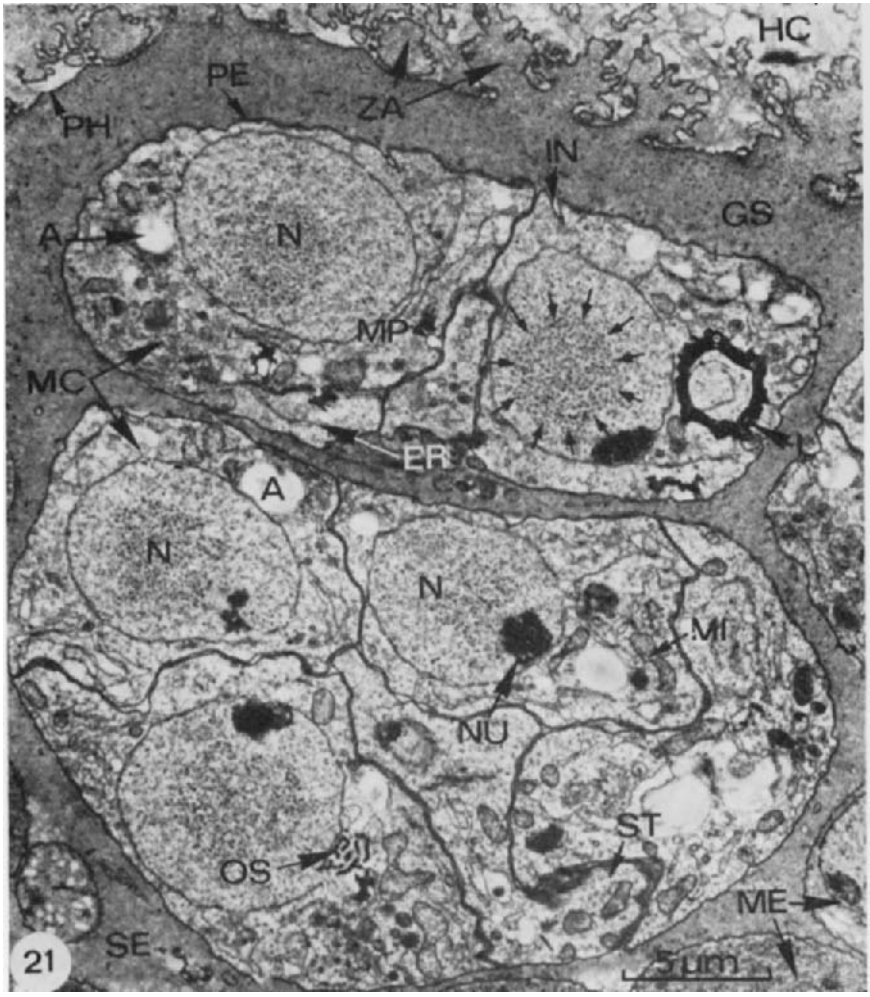


FIG. 21. Electron micrograph of *Sarcocystis tenella*. Section through the periphery of a cyst containing clusters of metrocytes.

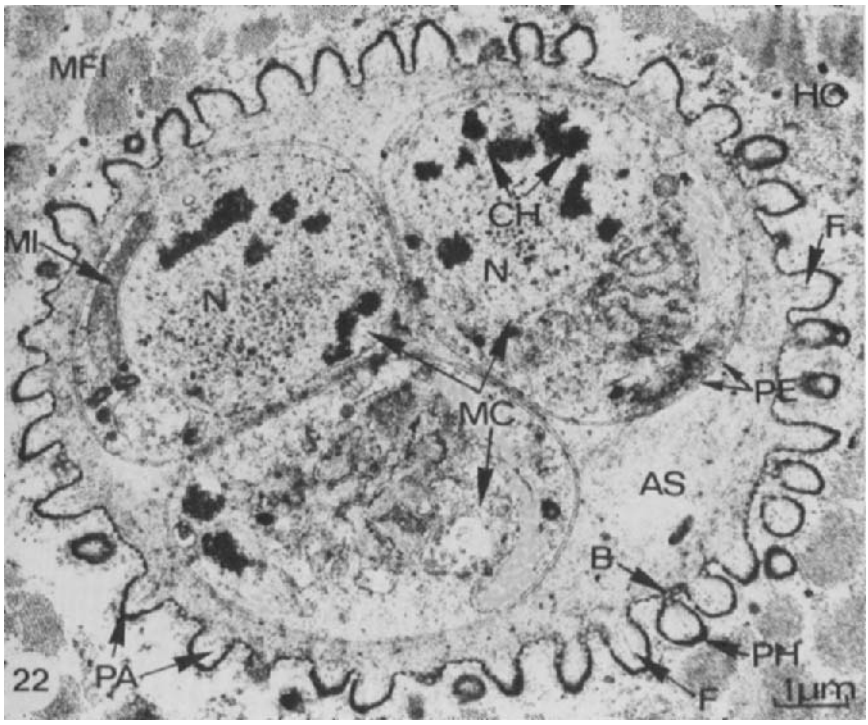
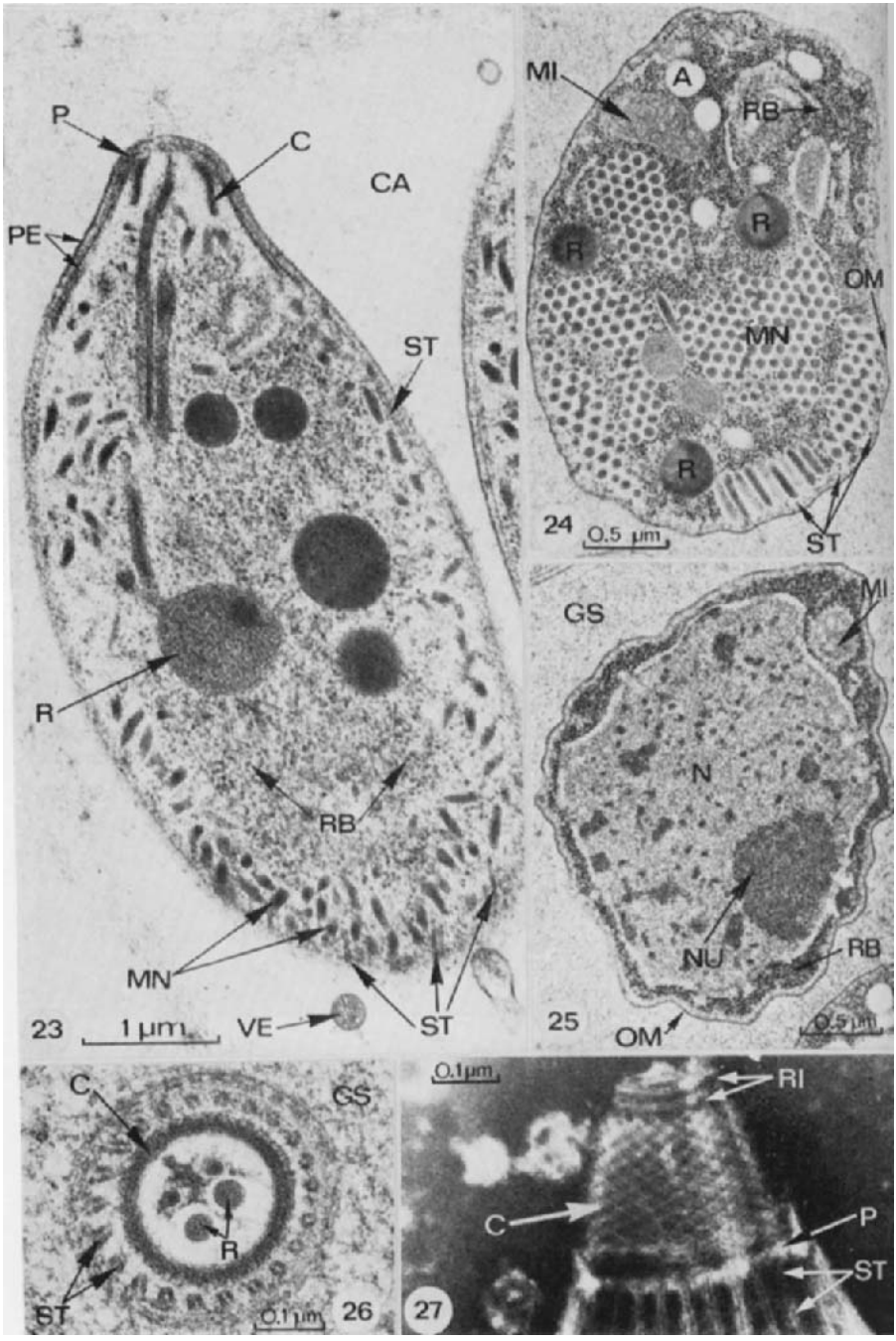


FIG. 22. Electron micrograph of *Sarcocystis bovihominis* from muscles of cattle. Section through a very young cyst containing three metrocytes.



FIGS 23–27. Electron micrographs of merozoites from cysts.
FIG. 23. *S. bovihominis* from cattle. Longitudinal section of the anterior pole.
FIGS 24–26. *S. tenella*. Cross sections.
FIG. 27. *S. tenella*. Negatively stained anterior pole with the protruded conoid. (From joint investigations with Dr. D'Haese and Prof. Peters.)

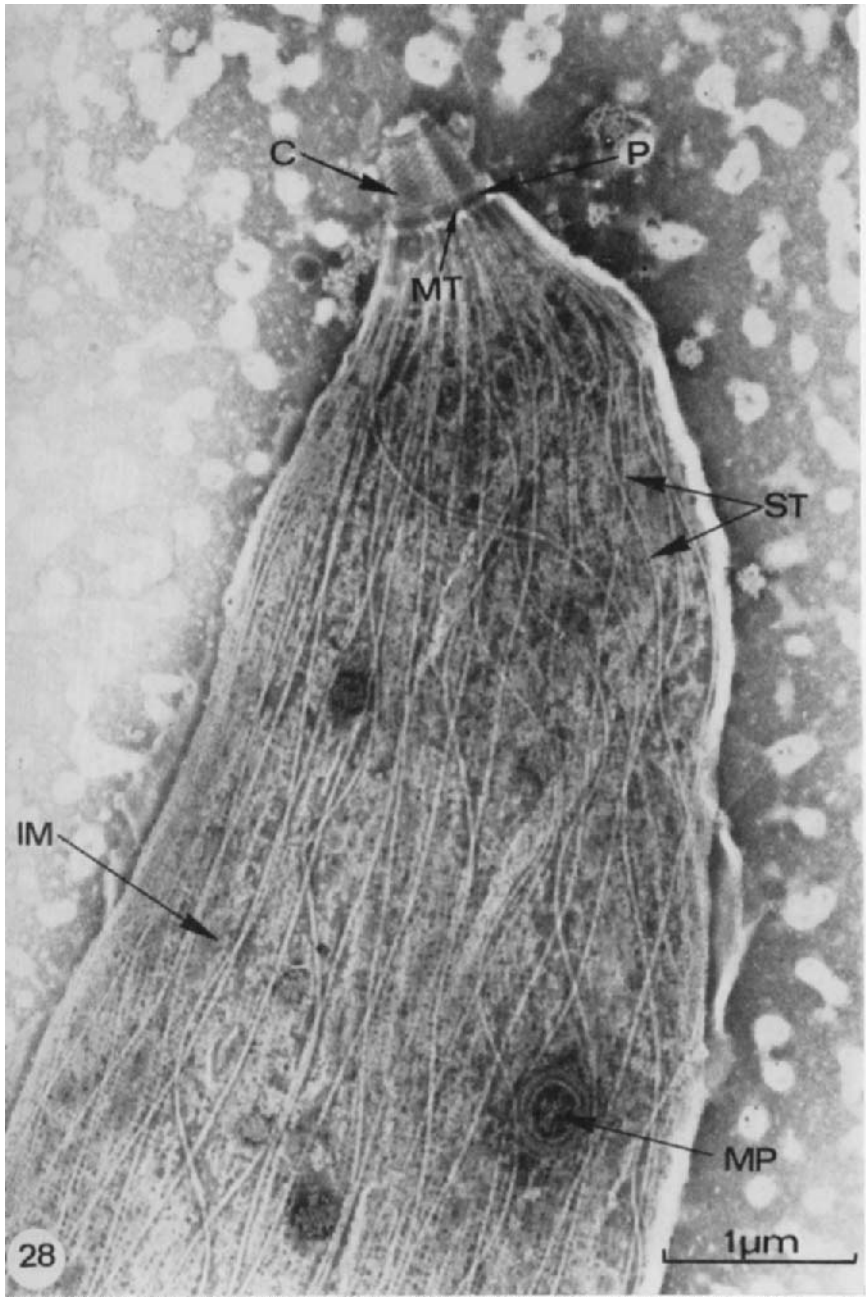
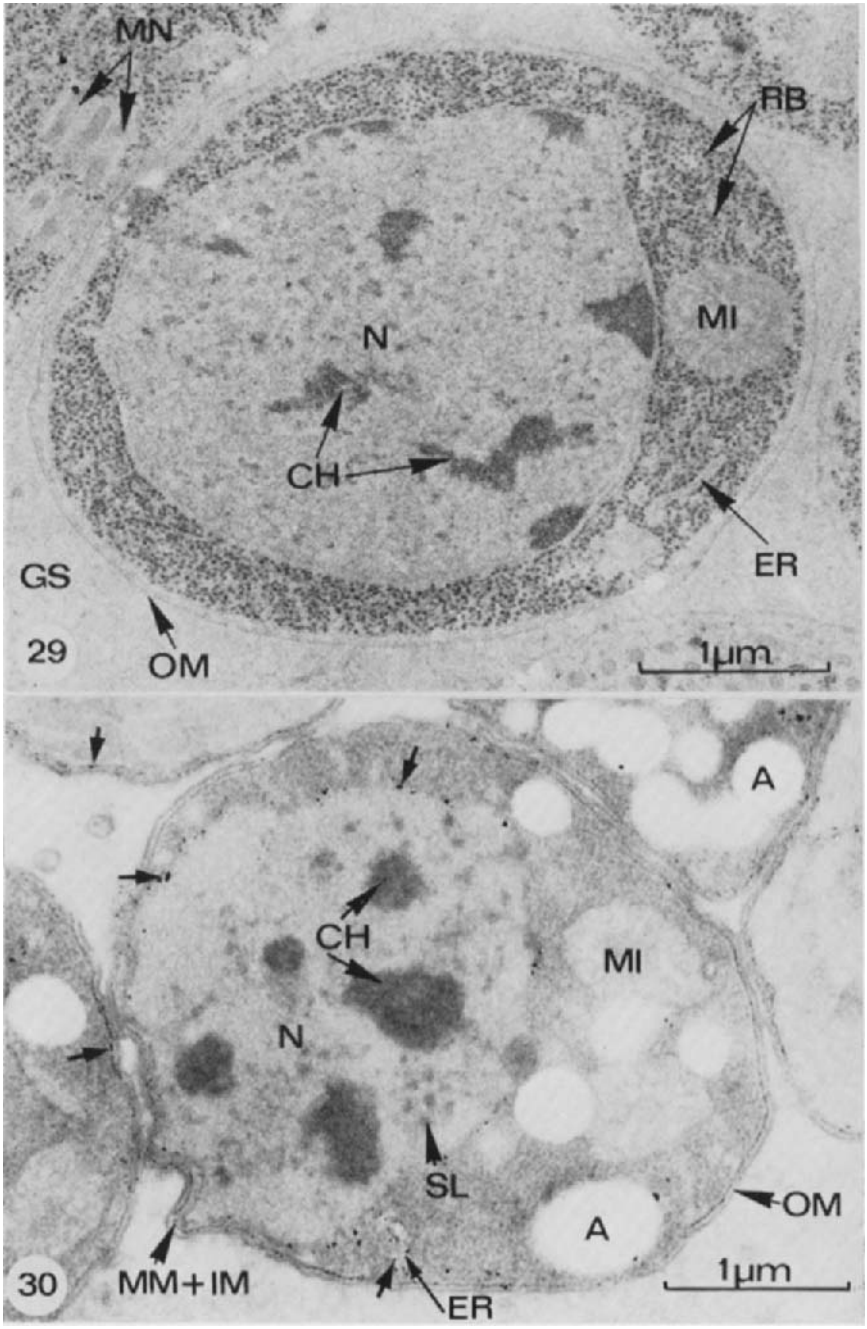


FIG. 28. Electron micrograph of a negatively stained merozoite from a cyst of *S. tenella*. Note the mesh-like pattern of the inner layers of the pellicle. (From joint investigations with Dr. D'Hease and Prof. Peters.)



FIGS 29–30. Electron micrographs of merozoites from cysts of *S. tenella*.
FIG. 29. Cross section of a merozoite treated with thallium to stain the DNA- and RNA-containing structures. (From joint investigations with Dr. Sénaud.)
FIG. 30. Cross section through a merozoite, where activity of ATP-ase is demonstrated.

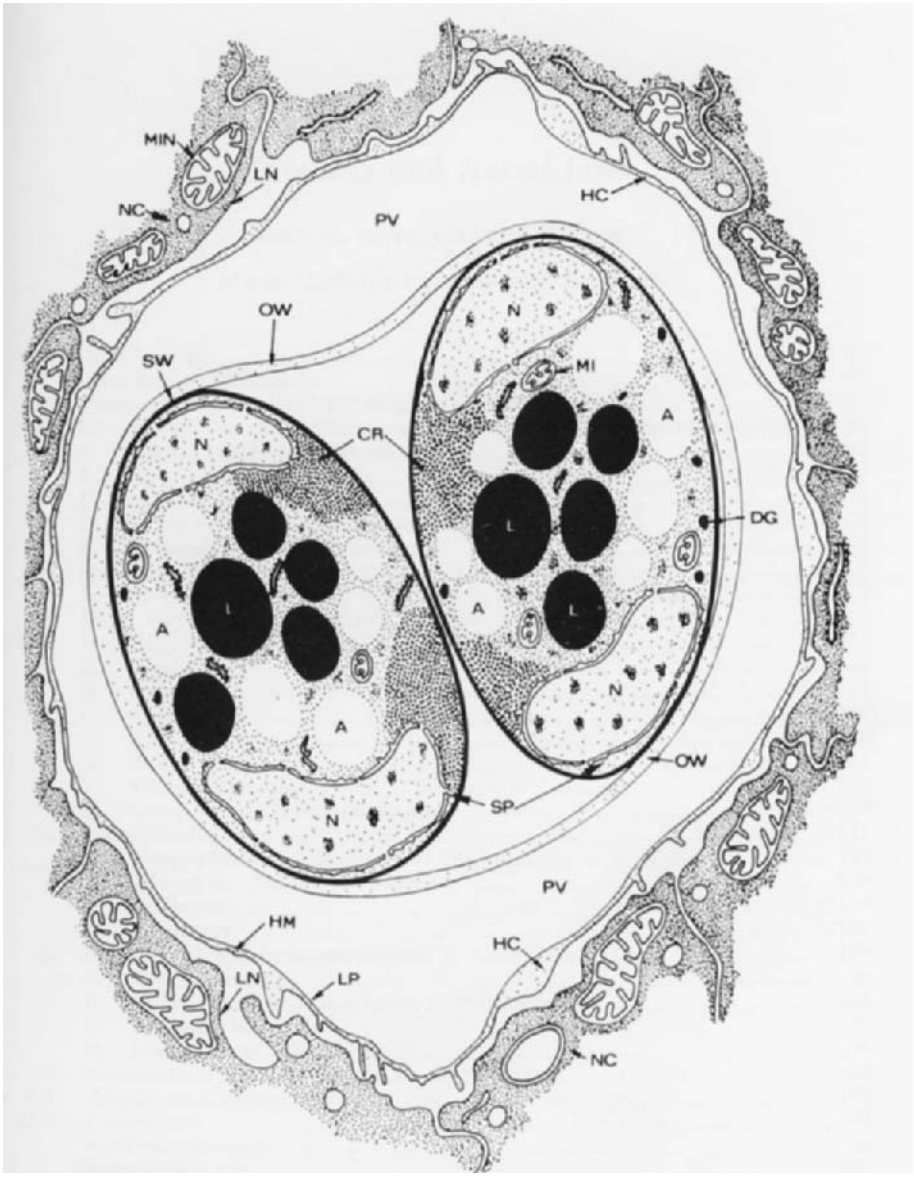


FIG. 31. *Sarcocystis tenella*. Section through a sporulating oocyst (diagrammatically). Note that the host cell is very small. The organelles seen within the two sporocysts were also present in unsporulated oocysts.

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Anisakis and Anisakiasis

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I. Introduction

An enormous literature has accumulated on the general biology of *Anisakis* Dujardin, 1845, and on the "accidental" infection of man and other animals with this nematode, a condition called anisakiasis. Certain other genera of

marine anisakine nematodes, including *Phocanema* (= *Terranova* = *Porrocaecum*) and *Contracaecum*, have also been implicated in human infection (see, for example, Jackson, 1975, Williams and Jones, 1976). These conditions have also been called anisakiasis (Cheng, 1976). However, this review is concerned only with *Anisakis sensu stricto* and with accidental infections associated with this genus.

This review has been restricted to *Anisakis* not only because members of this genus appear to be the most abundant of the marine anisakines but also because current knowledge indicates that they may be the most important with regard to human infection, at least on a worldwide basis.

That various developmental stages of *Anisakis* and other marine anisakines often occur together in the same individual hosts during the course of "normal" life histories has resulted in considerable taxonomic and other confusion. For this reason it has proved impossible in this review to avoid mention of other genera, but this may be rewarding in that available information on a related genus may help towards an understanding of the biology of *Anisakis*.

Larval stages of anisakine nematodes were probably first recognized in fish hosts as early as the 13th century (Myers, 1976). The period since 1945 has seen a vast increase in the output of relevant literature, for which there appear to be two main reasons.

First, economic anxiety concerning the occurrence of larval nematodes in the flesh of cod caught in Western North Atlantic waters and elsewhere led to scientific investigation of the biology and life history of anisakines in relation to populations of final hosts, with particular reference to *Phocanema* and seals (see, for example, Myers, 1960, and references therein).

Secondly, and most significantly with regard to the increase in the literature during the past 15 years or so, this period saw the first reports by Dutch workers (e.g. Van Thiel *et al.*, 1960) of a larval nematode from herring in the gastrointestinal tract of man in The Netherlands. At first the worm was incorrectly identified as "*Eustoma rotundatum*" but later corrected to *Anisakis* by Van Thiel (1962). This discovery prompted the formation in 1960 in The Netherlands of an "*Anisakis* Committee" to co-ordinate research (Ruitenberg, 1970). Subsequently an anisakiasis problem in man in Japan was recognized with the formation in that country in 1965 of a "research group on parasitic granuloma" which worked for three years with government aid (Oshima, 1972).

With the literature explosion that has accompanied these research activities it is not surprising that several review-type papers dealing wholly or partly with *Anisakis* and related genera have appeared in recent years, including those by Dollfus (1970), Ruitenberg (1970), Oshima (1972), Euzéby (1973), Van Thiel (1976) and Williams and Jones (1976). Certain aspects of anisakiasis in man, especially pathology and immunology, were reviewed by Areán (1971), Bisseru (1975) and Soulsby (1976). Five papers (i.e. those by Jackson, 1975; Myers, 1975; Cheng, 1976; Norris and Overstreet, 1976; Bier, 1976) were developed from presentations at a symposium held in New York in 1974 entitled "Anisakiasis: a new disease from raw fish."

The aim of this review is to bring together the many and varied aspects of

the extensive world literature on *Anisakis* and anisakiasis (*sensu stricto*), including use of the nematode as a biological tag in applied fishery science. Selectivity has been inevitable because the relevant literature is so enormous but we hope that no important information has been omitted.

II. Classification of *Anisakis*

The classification scheme for nematodes of the superfamily Ascaridoidea proposed by Hartwich (1974), based largely on features of the "excretory system", is adopted in this review. Keys to the higher taxa shown in the following complete classification of *Anisakis* are given by Anderson *et al.* (1974).

Class Nematoda
Subclass Secernentea
Order Ascaridida
Superfamily Ascaridoidea
Family Anisakidae
Subfamily Anisakinae
Tribe Anisakinea
Genus *Anisakis* Dujardin, 1845.

A clue to some aspects of the taxonomic confusion that exists in the literature regarding *Anisakis* Dujardin, 1845, lies in the following list of synonyms given by Hartwich (1974) for this genus:

Anisakis (*Skrjabinisakis*) Mozgovoi, 1951; *Capsularia* Zeder, 1800, *nec* Modeer, 1973; *Conocephalus* Diesing, 1861, *nec* Thunberg, 1812; *Filocapsularia* Deslongchamps, 1824, *nom. oblit.; Peritrachelius* Diesing, 1861; *Stomachus* Goeze in Zeder, 1800, *nom. nud.*

III. Taxonomy and Adult Morphology of *Anisakis*

In a revision of the genus *Anisakis*, Davey (1971a) gave the following generic diagnosis:

Three lips each bearing a bilobed anterior projection which carries the single dentigerous ridge; interlabia absent; excretory gland with duct opening between ventrolateral lips; oesophagus with anterior muscular portion (preventriculus) and posterior ventriculus, the latter being oblong and sometimes sigmoid or else as broad as long; no oesophageal appendix or intestinal caecum; vulva in middle or first third of body; spicules of male unequal; preanal papillae numerous; postanal papillae including a group of three or four pairs set close to the tip of the tail on the ventral side.

Habitat: Stomach and intestine of marine mammals.

Genotype: *Anisakis simplex* (Rudolphi, 1809, det. Krabbe, 1878).

Confronted with the descriptions, some very poor, of 21 species of the genus *Anisakis*, Davey (1971a) undertook critical examination of the extent of individual variation in taxonomic characters; he concluded that there are only three valid species (with 14 synonyms between them) and retained four others as *species inquirendae* for lack of sufficient data, as follows:

Valid species

1. *A. simplex* (Rudolphi, 1809, det. Krabbe, 1878) (with 10 synonyms).
2. *A. typica* (Diesing, 1860) (with one synonym).
3. *A. physeteris* Baylis, 1923 (with three synonyms).

Species inquirendae

1. *A. dussumierii* (van Beneden, 1870), from a dolphin in the Indian Ocean near the Maldive Islands.
2. *A. schupakovi* Mozgovoi, 1951, from Caspian seal in the Caspian Sea (redescribed and regarded as valid by Delyamure *et al.*, 1964—see also below).
3. *A. alexandri* Hsü and Hoeppli, 1933, from *Sotalia sinensis* in the (?) China Sea.
4. *A. insignis* (Diesing, 1851) from *Inia geoffrensis* in South American rivers (redescribed and regarded as valid by Petter, 1972—see also below).

The key provided by Davey (1971a) for the identification of adult *A. simplex*, *A. typica* and *A. physeteris* contains an error—two references are made to "oesophagus" when "ventriculus" is clearly intended. Davey claimed that the morphological characters in order of importance for identifying the three species named in his key are the spicules, the postanal papillae, the form of the ventriculus, the position of the vulva, and the form of the lips. These species are said to be satisfactory when discussed in geographical and ecological terms; the marine mammalian hosts are discussed in section V.

Kurochkin (1975) and, as mentioned earlier, Delyamure *et al.* (1964) redescribed *A. schupakovi* Mozgovoi, 1951, from Caspian seal (*Pusa caspica*) and regarded it as a valid species. Apparently Davey (1971a) was not acquainted with this redescription of *A. schupakovi* at the time of his revision of the genus; however, Kurochkin (1975) stated that "later Davey (personal communication) confirmed the validity of this species". On ecological grounds alone, *A. schupakovi* would appear to be valid in that its host (*P. caspica*) is a geographically isolated relict species confined to the Caspian Sea. Mokhayer (1974) referred to larval *A. schupakovi* in young sturgeons in the Caspian Sea.

Davey (1971a) did not refer to *Anisakis pacificus* Skryabin, 1959, from a sperm whale and other cetaceans in the northwestern Pacific Ocean; examination of the description of this species in relation to Davey's revision of the genus suggests that *A. pacificus* is a synonym of *A. simplex* (Dr. D. I. Gibson, personal communication). Oshmarin and Demshin (1972) described *A. rugocephalus* from "dolphins" in Vietnamese waters; the validity of this species is also doubtful and its possible synonymy with *A. simplex* should be investigated.

As mentioned above, *A. insignis* was regarded as a valid species by Petter (1972); her illustrated redescription, based on male and female specimens from *Inia geoffrensis* in the Rio Ibaré (Bolivia), ascribed certain morphological features to this species which have interesting implications for the generic

diagnosis given above and for the taxonomic criteria of Davey (1971a). These features as well as certain other aspects warrant further discussion.

According to Davey (1971a) the spicules of *Anisakis* spp. are unequal, the right one being consistently shorter than the left, and their ratio is useful taxonomically. However, Petter (1972) stated that the two thin alate spicules of *A. insignis* are identical. Furthermore, Petter referred to two cuticular folds forming two "superposed collars" at the base of the lips in *A. insignis*, distinctly visible in adult specimens but barely formed in young ones; this feature does not appear to have been reported for other species.

Despite Davey's (1971a) valuable discussion and revision of the genus, there still appear to be problems concerning the taxonomic distinction between *A. simplex* and *A. typica*. For example, Oshima (1972) reported that he was unable to distinguish the females of *A. typica* in blue–white dolphins (*Stenella caeruleoalba*) containing males of both *A. simplex* and *A. typica*. Furthermore, the possibility that eggs obtained from these female worms represent different "strains" adds further confusion (see Bier, 1976; and section IV. B).

Whilst Young and Lowe (1969) referred in the text of their paper to *A. typica* from marine mammals in British waters, confusion may arise if readers overlook a short erratum slip attached to this paper stating that "taxonomic studies by Davey (in press) suggest that the adult specimens of *Anisakis* recorded in this paper are probably *Anisakis simplex* and not *Anisakis typica*".

Nomenclatural instability has also added to problems of the taxonomic status of *A. simplex*. Van Thiel (1966) proposed the name "*Anisakis marina* (Linnaeus, 1767)" for the species occurring in marine mammals in the North Sea and South Atlantic and this name occurs in more recent literature (e.g. Ruitenberg, 1970). However, following the arguments put forward by various workers, including Khalil (1969) and Davey (1971a), Van Thiel (1976) agreed to use the name *A. simplex* (and not *A. marina*) to avoid confusion.

With regard to adult morphology, Grabda (1976) has recently provided adequate illustrated descriptions of adult male and female *A. simplex*, including cross-section morphology at different levels, so the following account is restricted to certain morphological features which, among others, may have received inadequate attention.

Inglis (1964a) described and illustrated the cephalic septum and lips of *A. simplex, A. physeteris* and other ascaridoids; he argued that the septum enables lip movements to be controlled largely by muscles of the oesophagus with some assistance from the body-wall muscles which, in *Anisakis*, are of the polymyarian—coelomyarian type.

A comparative study of the complex structure of the basically three-layered cuticle in *A. simplex, A. physeteris* and other ascaridoids was also made by Inglis (1964b). The cuticles of the two *Anisakis* species differed in several details including topography of the outer surface. In *A. simplex*, the outer surface (apart from the anterior tip of the body) is highly modified by the development of large posteriorly overlapping, flanged annulations; this feature is not apparent in *A. physeteris*.

Davey (1971a) referred to a pair of cervical papillae near the anterior end of the body as characteristic of all species of *Anisakis*, whilst Petter (1972) described two lateral deirids slightly below the nerve ring in *A. insignis*. Beverley-Burton *et al.* (1977) mentioned lateral cervical papillae about 0·3 mm behind the nerve ring in larval *A. simplex*. McLaren (1976) stated that cervical papillae and deirids are different names for the same sense organs and speculated that they may be responsible for determining whether or not the nematode can pass successfully through a restricted space.

A mucron (tail spine) at the tip of the tail has been claimed to be "a larval feature normally lost at the final moult" (Davey, 1971a) but not all "larval types" of *Anisakis* appear to possess one (see section IV. E). According to Beverley-Burton *et al.* (1977) the mucron of larval *A. simplex* (a type (I) larva) is retractable and it is tempting to suggest, therefore, that it functions in penetration but evidence is lacking. Davey (1971a) stated that adult female *Anisakis* sometimes possesses what looks like a mucron, sunk back into a fold of cuticle, but that adult males never have such a structure.

With regard to certain aspects of the anterior part of the alimentary tract of adult *Anisakis*, Davey (1971a) reported that the junction of the preventriculus with the ventriculus (component parts of the oesophagus) is suggestive of a sphincter-like arrangement in all species; the feeding mechanism of *Anisakis* has not been studied in detail so the function of the supposed sphincter is unclear. According to Davey (1971a), the ventriculus in *A. simplex* is often, but not invariably, sigmoid and always longer than it is broad; that of *A. typica* is essentially similar. The ventriculus of *A. physeteris* is short, never sigmoid and may be broader than long; the short form of the ventriculus appears to have given rise to the assumption that *Anisakis* larva (II) develops into this species (see also section IV. E2).

IV. Life History, General Development and Larval Morphology of *Anisakis*

A. General life-history pattern

According to various authors (e.g. Chitwood, 1974) the life history of a "typical" nematode shows the following course of development characterized by four moults:

Egg—First stage larva—(1st moult)—Second stage larva—(2nd moult)—Third stage larva—(3rd moult)—Fourth stage larva—(4th moult)—Fifth stage: adult.

The term "larva" is used here because of its extensive usage in the literature, although the terms "juvenile" (Hyman, 1951) or "nymph" (Chitwood, 1974) may be more appropriate. Various adaptations or modifications to this basic sequence are seen in different groups of nematodes and such is the case with *Anisakis* and other ascaridoids.

Before embarking on a discussion of the life history of *Anisakis* and its developmental stages, it should be pointed out that problems of semantics and terminology feature among the many difficulties associated with interpretation

of the literature on this nematode genus and its relatives. The fourth-stage larva of *Anisakis* is often referred to as a "preadult", whilst McClelland and Ronald (1974b) in an account of *Contracaecum osculatum* refer to this stage as a "subadult" and use "preadult" for the fifth stage (presumably an immature adult). Other terms used loosely in the relevant literature include "exsheathment", and the "transfer", "transport", "paratenic" and "intermediate" host concepts but it would be invidious to particularize. Odening (1976) drew attention to the need for clarification of concepts and terminology of hosts and parasites and attempted this in a lengthy account.

Field observations indicate that crustaceans (especially euphausiids), squid and fish (especially teleosts) are hosts of *Anisakis* larvae, whilst marine mammals (especially cetaceans) harbour larvae and egg-producing adults. The highly speculative scheme for the life history of *Anisakis* given in Fig. 1 is based on an appraisal of the relevant literature; it is intended as a companion to the following discussion and is offered with due reserve. The reader's attention is also directed to the pictorial representations of the life history given by Oshima (1972) and by Williams and Jones (1976).

The hosts referred to in Fig. 1 may be regarded as "natural" hosts featuring in the course of the "normal" life history of *Anisakis*. Other animals, including man, may be regarded as "accidental" hosts which acquire infection by eating fish or squid harbouring live *Anisakis* larvae; such larvae either do not develop at all or may develop to the preadult (fourth) stage—in either case they ultimately die and represent a loss to the parasite population. Various other aspects of accidental infections are dealt with elsewhere in this review.

With regard to the "normal" life history, there are difficulties in assigning a role to the various hosts named in Fig. 1 and to assessing the relative importance of each, particularly in view of the paucity of field observations and the lack of experimental evidence.

The first moult evidently takes place within the egg; the larva which hatches is second stage and bears the cuticle of the first stage as a sheath (Smith, 1971; Van Banning, 1971; Oshima, 1972). There is evidence that *Anisakis* undergoes two moults in the marine mammalian final host (Kagei *et al.*, 1967; Kikuchi *et al.*, 1967; Jacobsen and Berland, 1969; Gibson, 1970), presumably the 3rd and 4th. If these observations are correct, three of the four moults of a "typical" nematode are accounted for (i.e. the 1st, 3rd and 4th moults) but what of the remaining (2nd) moult? It has been assumed by authors that it is the third-stage larva of *Anisakis* which is infective to the final host and that this stage occurs in fish and squid. This implies that a second moult has taken place, but in which host? Some Japanese workers (e.g. Oshima, 1972) are convinced that a moult occurs in a crustacean (euphausiid) host but experimental proof is lacking. Other workers (e.g. Kagei, 1969) indicated that the moult takes place in fish but again without experimental demonstration. Yet others are non-committal or obscure on this point. Larvae from crustaceans, squid and fish collected in the field are morphologically similar but detailed morphological comparisons of larvae from these different sources are lacking.

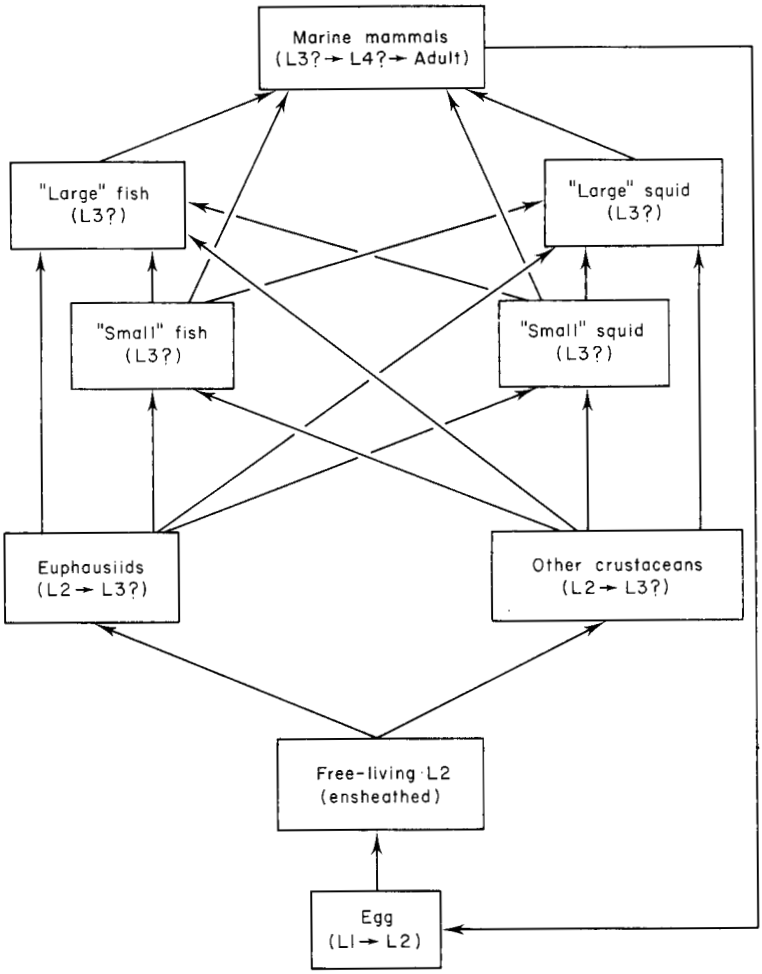

FIG. 1. Speculative life-history pattern of *Anisakis*.

What little evidence there is suggests that larvae in crustaceans (or at least in euphausiids) grow from less than 0·33 mm up to 30 mm or more in length and it might be argued that such length increase with its accompanying increase in girth is unlikely to occur without the intervention of a moult.

If a moult does occur in crustaceans then these are "intermediate" hosts, whilst fish and squid are "paratenic" hosts. If this is the case, then larvae from crustaceans might be expected to be directly infective to final hosts (a possibility not shown in Fig. 1)—unless it is argued that "conditioning" of larvae in fish or squid is required before they are infective.

Experimental evidence that larvae are capable of transferring from fish to fish in prey–predator relationships without apparent morphological change (Smith, 1974) perhaps indicates that a moult does not occur in fish. Sluiters (1974) reported that *Anisakis* larvae from the stomach lumen of herring were consistently smaller than those recovered from the body-cavity of this host; this observation and Sluiters' conclusion that larvae grow in herring require confirmation. In-vitro observations by, for example, Sommerville and Davey (1976) and Grabda (1976) on larvae from fish indicate that this stage does not feed; apparently feeding does not begin until after the moult to the preadult (fourth) stage.

Published evidence concerning the life histories of certain other aquatic ascaridoid nematodes is not especially helpful in interpretating that of *Anisakis*. For example, McClelland and Ronald (1974a,b) compare the results of their in-vitro culture work on *Terranova* (= *Phocanema*) *decipiens* and *Contracaecum osculatum* with the field observations of other workers; eggs were used to initiate the cultures. They suggest that these nematodes may develop "neotenically" from second stage to infective stage under natural conditions—the infective stage being third stage in the case of *C. osculatum* but fourth stage in the case of *P. decipiens*. Intriguing but unresolved problems of this nature are discussed by Cheng (1976) and the reader's attention is also directed to the life history accounts by Norris and Overstreet (1976) for *Thynnascaris* spp. of fish and by Huizinga (1966, 1967) for *Contracaecum* spp. of piscivorous birds.

Work with *Anisakis in vitro* has been restricted largely to cultures initiated with larvae from fish (see section VI). It would be of great value to undertake culture work starting with the egg and to couple this approach with a study of experimental infection in crustaceans.

Information on the morphology and general biology of the egg, second-stage larva and of the larval stages of *Anisakis* in crustaceans, squid and fish is given in the following sections.

B. EGG

Adult female *Anisakis* recovered from blue-white dolphins in Japanese waters by Kobayashi *et al.* (1966, 1968a) were associated with male worms, most of which were identified as *A. simplex* but some as *A. typica*. According to Oshima (1972) the female worms were of two morphological types, one slender bodied with the vulva in the anterior half of the body, the other stout bodied with the vulva in the posterior half. Both female types yielded morphologically indistinguishable ellipsoidal eggs measuring 45·5–58·1 × 41·3–53·2 μm which, in terms of time required for hatching at various temperatures from 2 to 27°C, appeared to represent either of two "strains"—one adapted to high temperatures, the other to low temperatures. As Bier (1976) has indicated, this finding "clouds the already confused taxonomic distinction between *A. simplex* and *A. typica* which Davey (1971a) had seemingly resolved by associating *A. typica*, the species of the stout females, with warmer climates".

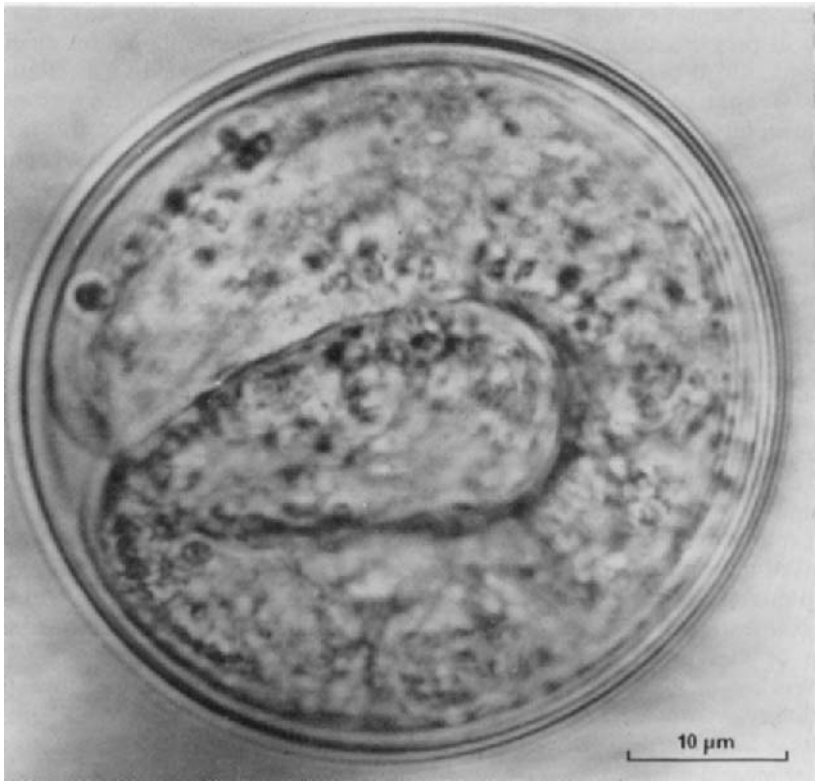

FIG. 2. Embryonated egg of *Anisakis* (bar scale, 10 µm).

The worms cultured *in vitro* by Van Banning (1971) and subsequently identified as *A. simplex* by Pippy and Van Banning (1975) produced eggs round to oval in shape and measuring 40×50 µm on average. These eggs hatched in 4–8 days at 13–18°C and in 20–27 days at 5–7°C.

The eggs are apparently passed unembryonated with the faeces of marine mammals and subsequently embryonate in sea water (Fig. 2). According to Sluiters (1974) the eggs of *Anisakis* sink in sea water and may reach the seabed by the time of hatching; however, the rate of sinking will depend on local conditions, e.g. upwelling of currents, and the hatch time evidently depends on temperature.

C. SECOND-STAGE LARVA

The free-living larva which emerges from the egg is apparently a second stage and ensheathed in the cast cuticle of the first moult which evidently takes place within the egg (Fig. 3). The larval body narrows posteriorly, shows a nerve ring and bears an anterior boring tooth, whilst the alimentary

tract is poorly differentiated (Van Banning, 1971; Oshima, 1972) but further detailed information is lacking. Average length without the sheath is given as 230 μm by Van Banning (1971) and as 286 μm by Oshima (1972); average length with the sheath is 355 μm according to Van Banning. Smith (1971) gives a length range of about 0·22–0·29 mm without the sheath, and of about 0·33–0·37 mm with the sheath. Larvae are very active and survive without exsheathing in sea water for three to four weeks at 13–18°C, and for six to seven weeks at 5–7°C. Increasing mortality was seen at temperatures above 20°C and 34°C was lethal (Van Banning, 1971).

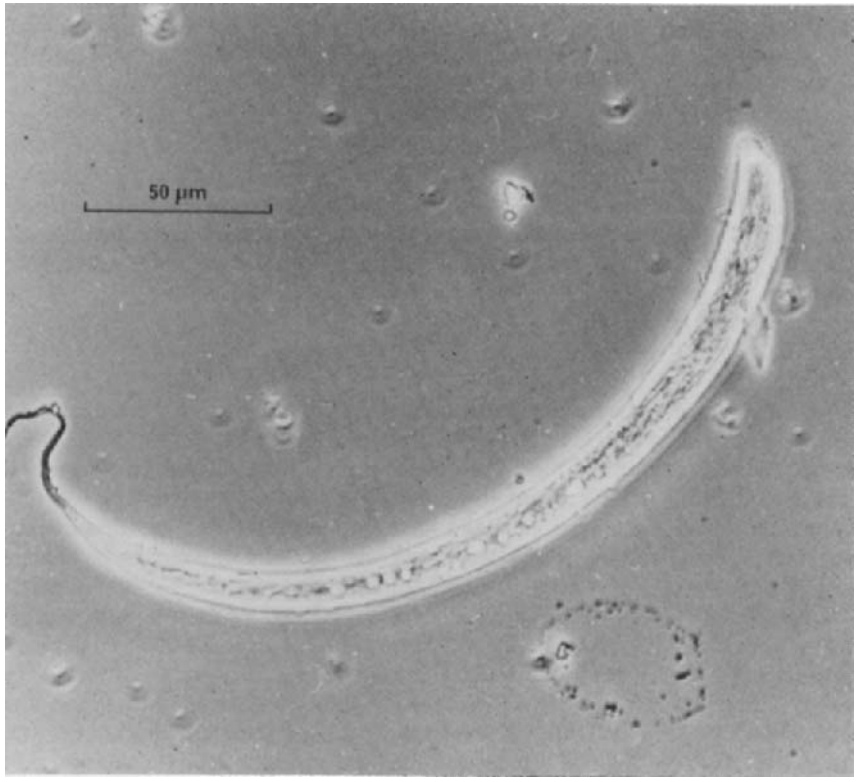

FIG. 3. Free-living, ensheathed second-stage larva of *Anisakis* (bar scale, 50 μm).

With regard to the fate of hatched larvae, workers have been influenced by a) the apparent importance of crustaceans in the life histories of other aquatic ascaridoids and b) the importance of crustaceans in the diet of fish heavily infected with *Anisakis* larvae. These factors have led to field surveys for larval *Anisakis* in marine crustaceans (see section IV. D). However, there is little experimental evidence concerning the mode of entry into crustaceans or subsequent development of the ensheathed larvae and further work is

necessary. Japanese workers (Oshima, 1969; Oshima *et al.*, 1968; Kagei, 1969) have attempted experimental infection of euphausiids, and their results are summarized by Oshima (1972). In two experiments, ten *Euphausia similis* and six *E. pacifica* were exposed singly to large numbers of hatched larvae and killed at intervals of 1–8 days after exposure. The eggs from which the larvae were hatched originated from female worms associated with males identified as *A. simplex* (mostly) with some *A. typica*. Interpretation of the tabulated results suggests that six of the 16 euphausiids became infected with a total of 18 larvae, of which nine had exsheathed. The larvae were found in the haemocoel of the euphausiids and the data indicate that at least some multiple infections occurred. The lengths and widths of the larvae, which had spent varying periods up to eight days in euphausiids, fell within the range of those for freshly-hatched larvae; the data are not strictly comparable because different methods of fixation were used. The observations could not be continued because no euphausiid survived beyond eight days. Lasker and Theilacker (1965) drew attention to the problems of maintaining euphausiids alive in the laboratory for long periods. They suggest a method for capturing undamaged euphausiids which are then nurtured individually in the laboratory and each offered several thousand newly hatched *Artemia salina* nauplii. Such an approach may yield suitable experimental material for work with *Anisakis*.

D. LARVAE IN MARINE CRUSTACEANS

As indicated above, field surveys of marine crustaceans for *Anisakis* have been conducted by several authors with some success (Table 1). The crustaceans referred to in this table are all of the subclass Malacostraca and represent the following orders: Euphausiacea (*Thysanoessa, Meganyctiphanes* and *Euphausia*), Decapoda (the caridean shrimp, *Pandalus* and the brachyuran crab, *Hyas*) and Amphipoda (*Caprella*). Thus *Anisakis* appears to be loosely host specific even at this stage of the life history.

Table 1 refers only to surveys which have yielded positive results. Kagei (1974) tabulated the results of an examination of over 67 000 crustaceans (representing three different orders) collected in different years from 19 widely scattered localities in the Antarctic Ocean, waters around Japan, the East China Sea, Sea of Okhotsk, Northern North Pacific and Bering Sea. Of these, only two euphausiids from one locality harboured larval *Anisakis*. In the smaller area of the Northern North Sea and European North Atlantic, Smith (1971) found a patchy distribution of larval *Anisakis* in euphausiids with infection apparently absent at many localities. Collard (1970) stated that "Brinton (pers. comm.) in his exhaustive studies of the Euphausiacea . . . noted that nematode larvae are not abundant in these caustaceans (*sic*), and the author failed to find them in any of several thousand examinations". Noble (1973) remarked that he "recently examined 983 euphausiids taken from Californian coastal waters and not one was infected with nematodes or any other metazoan parasite".

The few surveys conducted so far indicate a low prevalence of infection in

TABLE 1

Anisakis *larvae in marine crustaceans*

Locality	Hosts	No. examined	No. infected (% infected)	References
Barents Sea	*Caprella septentrionalis*	855	1 (0·117)	Uspenskaya (1963)
	Hyas araneus	990	1 (0·101)	
	Thysanoessa raschii	?	1	
European North Atlantic and Northern North Sea	*Thysanoessa inermis*	2730	18 (0·659)	Smith (1971)
	T. longicaudata	950	3 (0·316)	
	Meganyctiphanes norvegicus	3178	1 (0·031)	
Bristol Bay (Bering Sea) and Northern North Pacific	*Thysanoessa raschii*	121	3 (2·479)	Shimazu and Oshima (1972)
	T. longipes	438	3 (0·685)	
	Euphausia pacifica	54000	1 (0·002)	
East China Sea	*Euphausia pacifica*	28219	2 (0·007)	Kagei (1974)
Sea of Japan	*Pandalus borealis*	5046	5 (0·099)	Shiraki *et al.* (1976)
Sea of Okhotsk	*Pandalus kessleri*	724	2 (0·276)	Shiraki *et al.* (1976)

crustaceans (Table 1) and only one larva per infected host; this may not be inconsistent with the known abundance of *Anisakis* larvae in fish for the following reasons. With regard to euphausiids, for example, the population density is often high and they are eaten in enormous quantities by fish (Mauchline and Fisher, 1969), which may therefore acquire large numbers of larvae despite the low prevalence of infection in euphausiids. Furthermore, larvae are apparently long-lived in fish and therefore tend to accumulate with increasing host age (e.g. Bishop and Margolis, 1955; Davey, 1972a) and larvae may transfer from fish to fish in prey–predator relationships (Smith, 1974). It should be stressed that whilst present evidence suggests that euphausiids are of importance among the crustacean hosts of *Anisakis*, further work is necessary to assess the relative importance of these and other invertebrates in the life history. No detailed morphological study of larvae from crustaceans is available. The 41 larvae reported so far ranged in length from 5·1 mm (Smith, 1971) to 32·7 mm (Oshima *et al.*, 1969). In addition to these 41 larvae, Sluiters (1974) referred to an *Anisakis* larva, 9·4 mm long, which was apparently emerging from a euphausiid, *Thysanoessa raschii*, in the stomach of a North Sea herring. In general morphology (especially in relation to characteristics of the ventriculus and tail) all 42 larvae appear to resemble *Anisakis* larva (I) of Shiraki (1974) and *Anisakis* sp. larva (I) of Berland (1961) from fish and squid (see section IV. E1).

Reimer *et al.* (1971) referred to larvae of *Anisakis* from chaetognaths in the North Sea as being (in translation) "typical for the genus in the structure of the powerful oesophageal bulb without caeca"; this brief description lacks an illustration and may fit that of some other nematode genus (possibly not even an ascaridoid). These authors also stated that "Uspenskaya (1963) indicates that larvae of *Anisakis* are found in euphausiids and chaetognaths" but we can find no reference to larval *Anisakis* in chaetognaths in Uspenskaya (1963) or in Uspenskaya (1955, 1960).

In Uspenskaya (1963) there is an illustrated description of a larval "*Eustoma rotandatum* (Rudolphi, 1819)" from the anomuran decapod *Lithodes maja* in the Barents Sea which appears to resemble larval *Anisakis*. Vivares (1972/73, 1973) also referred to *Eustoma rotundatum* (without illustration) in three decapod crustaceans in the Mediterranean. The status of these forms is uncertain, especially since the names "*E. rotundatum*" or "*E. rotundata*" are implicated in the confused taxonomy and biology of another ascaridoid genus, namely, *Pseudanisakis* (see, for example, Williams and Richards, 1968; Gibson, 1973, 1974).

E. LARVAE IN MARINE FISH AND SQUID

The abundant literature on larval anisakids (and other ascaridoids) in marine fish and squid is complicated by a variety of taxonomic and nomenclatural problems, many of which remain unresolved. The state of this confusion with regard to larvae in fish may be judged from the papers by, for example, Punt (1941), Baylis (1944), Dollfus (1953, 1970), Templeman *et al.* (1957), Berland (1961) and Myers (1975) and with reference to the

many earlier papers cited by these authors. Although a detailed account of these problems is outside the scope of this review, it may be helpful to point out that complications have arisen from attempts to identify larval nematodes to genus or even species on somewhat tenuous morphological grounds, despite lack of knowledge of the life history and the absence of experimental evidence.

According to Myers (1975) the literature since 1767 contains over 100 descriptions of anisakine larval stages, the classification of which is too often based on arbitrary measurements. On the basis of anterior digestive tract formation and position of the "excretory" pore, Myers (1975) recognized five "larval types" to which generic names are applied loosely, viz. *Anisakis* (*sensu lato*) larvae, *Phocanema* (*sensu lato*) larvae, *Contracaecum* (*sensu lato*) larvae, *Raphidascaris* (*sensu lato*) larvae and *Multicaecum* (*sensu lato*) larvae.

Hartwich (1974) proposed a classification of adult nematodes of the superfamily Ascaridoidea based largely on the "excretory system". If the Hartwich scheme reflects phylogenetic relationships (as it is claimed to do), then the groupings and subgroupings of genera under the "larval types" proposed by Myers appear highly artificial in that genera from different families, subfamilies or tribes are associated together. For example, with regard to the topic of this review, under *Anisakis* (*sensu lato*) larvae is a subgroup comprising *Anisakis* (Anisakidae: Anisakinae: Anisakinea), *Paranisakiopsis* (Anisakidae: Raphidascaridinae: Paranisakinea) and *Acanthocheilus* (Acanthocheilidae), characterized by a ventriculus lacking both intestinal caecum and with the "excretory" pore opening at the base of the subventral lips. (It is relevant to mention that Gibson and Taylor in 1976 suggested transfer of *Paranisakiopsis* to Anisakinae.)

In a detailed account of the morphology and morphometrics of larval anisakids in fish and squid from northern coastal waters of Japan, Shiraki (1974) recognized four "larval types" of *Anisakis*, namely *Anisakis* larva (I), (II), (III) and (IV), along with other larval types representing *Terranova* (= *Phocanema*), *Contracaecum, Raphidascaris* and *Thynnascaris*; much of the Japanese work in this field during the past decade or so was reviewed.

Shiraki's "larval types" of *Anisakis* fall into the *Anisakis* (*sensu lato*) larvae group (*Anisakis—Paranisakiopsis—Acanthocheilus* subgroup) of Myers (1975) but, in view of the problems outlined above, further comment is not possible. The following discussion of Shiraki's "larval types" in relation to the *Anisakis* larvae reported by European and other workers is presented with some reserve.

The diagrammatic representations of the anterior and the posterior end of an *Anisakis* larva given in Fig. 4 are intended to facilitate discussion. The descriptive terms used for the anterior part of the alimentary tract are those of Myers (1975). With regard to descriptive terms, the following variations are among those which occur in the literature and which may lead to confusion: oesophagus (= pharynx); preventriculus (= muscular oesophagus); ventriculus (= glandular oesophagus). Furthermore, the measurements given for the oesophagus by some workers appear to exclude the ventriculus.

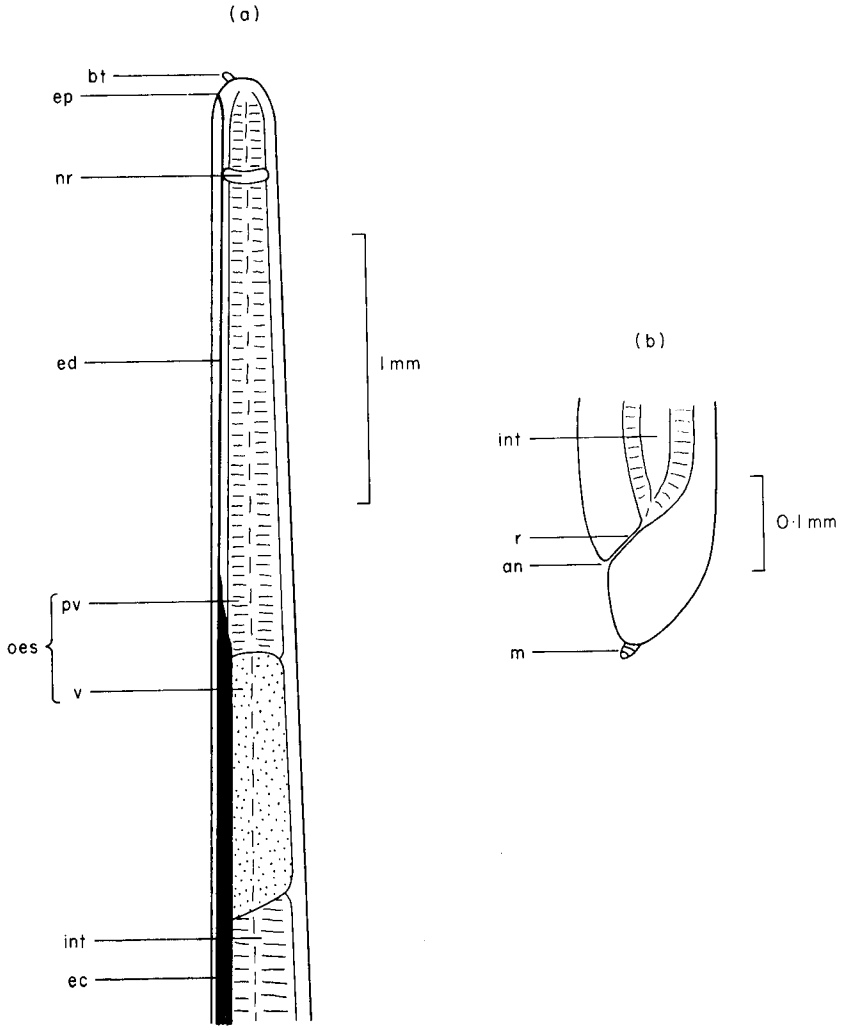

FIG. 4. Diagram of larval *Anisakis* from fish (a) anterior end (bar scale, 1 mm), (b) posterior extremity (bar scale, 0·1 mm)—anal glands omitted. Anus, an; boring tooth, bt; "excretory" cell, ec; "excretory" duct, ed; "excretory" pore, ep; intestine, int; mucron, m; nerve ring, nr; oesophagus, oes; preventriculus, pv; rectum, r; ventriculus, v.

Japanese and other workers have given detailed ratios of the length of various parts of the body to total body length for larval *Anisakis* (and other larval anisakids); such ratios are not considered in this review because they may be of value only if account is taken of allometric growth in nematodes (Inglis, 1954) and if appropriate morphometric analyses are undertaken (see, for example, the approach used by McClelland and Ronald, 1974a,b).

1. Anisakis *larva* (*I*)

This larval type is described by Shiraki (1974), Oshima (1972) and Koyama *et al.* (1969) as possessing a relatively long ventriculus with an oblique ventricular-intestinal junction, and a rounded tail bearing a mucron.

The head bears one dorsal and two ventrolateral lips; between the ventrolateral lips is an anteroventrally directed boring tooth, just behind which opens the "excretory" pore. Some morphometric data are given in Table 2.

Japanese workers regard this larval type as identical to *Anisakis* sp. larva (I) of Berland (1961) from teleosts in Norwegian waters. Such larvae from herring in the northern North Sea were cultured *in vitro* to the adult stage by Van Banning (1971) and identified as *Anisakis simplex* (Rudolphi, 1809, det. Krabbe, 1878) by Pippy and Van Banning (1975).

The recent illustrated description of the larva by Beverley-Burton *et al.* (1977), including a comparison with the earlier descriptions by Berland (1961), Grainger (1959), Punt (1941) and earlier workers, provides some interesting new information on aspects of morphology; unfortunately, comparable detailed information is not available for other larval types. The "excretory cell" shows anterior extensions of the nucleus and a bifurcation of the duct in its narrow posterior part; neither of these features was mentioned by Lee *et al.* (1973) in their ultrastructural study of the "excretory system" of unspecified *Anisakis* larvae, or by Mueller (1927) in his study of the adult *A. simplex* system. The "excretory cell" lies ventrally and somewhat to the left side in the anterior region of the pseudocoel; it is elongate to oval in cross section, except at the extremities where it is rounded, and is contiguous to hypodermal cells, alimentary canal and, occasionally, somatic musculature. In the intestinal region the cell shows a connection with the left lateral chord. The presence of one dorsal and two small ventrolateral oesophageal glands confirms Hsü's (1933) observations on adult *Anisakis*. The terminal tail spine (mucron) is said to be retractable. The observation of three rectal (anal) glands (two dorsal, one ventral) is interesting in relation to the two rectal glands reported by Sluiters (1974) in an *Anisakis* larva (lacking a mucron and apparently moulting) which appeared to be emerging from a euphausiid in a herring stomach. Sluiters' observations are relevant to the controversy surrounding the timing and place of the supposed second moult in *Anisakis* (see also Section IV. A) but require confirmation; the position of rectal glands as illustrated by some authors indicates a possible confusion with the four epithelial cells which, according to Beverley-Burton *et al.* (1977), externally line the short intestinal-rectal valve.

That larvae from herring and salmon at widely separated localities in the North Atlantic and North Sea are morphologically similar suggested to Beverley-Burton *et al.* (1977) that Type I larvae represent a single species. However, genetic heterogeneity (acid phosphatase polymorphism) among the different samples suggested the presence of either more than one species or more than one population of the same species. Involvement of larval *A. physeteris* was discounted because "larval *A. physeteris* (= *Anisakis* Type II larvae) are likely morphologically different from Type I larvae (Oshima,

TABLE 2

Some morphometric features of four "larval types" of Anisakis *from northern coastal waters of Japan. Mean: range measurements (in mm) in each case. (Taken from Shiraki, 1974)*

Larval type	No. of specimens	Total length	Maximum Width	Preventriculus length	Ventriculus length	Tail length	Host(s)
(I)	9	28·4:23·0–31·7	0·49:0·45–0·52	2·06:1·76–2·33	1·30:1·08–1·46	0·12:0·10–0·13	Teleosts and squid
(II)	13	25·7:21·6–31·9	0·61:0·53–0·71	2·15:1·80–2·51	0·58:0·47–0·71	0·28:0·21–0·38	Teleosts and squid
(III)	19	28·9:23·8–38·4	0·84:0·65–0·97	1·83:1·46–2·33	0·57:0·47–0·73	0·16:0·10–0·20	Teleosts
(IV)	9	20·1:14·8–26·4	0·54:0·41–0·67	1·59:1·24–2·18	0·38:0·30–0·59	0·15:0·11–0·19	Teleost (*Theragra chalcogramma*)

1972)". Larval *A. simplex* and *A. typica* may be morphologically and morphometrically similar but from a consideration of the supposed geographic range of the two species (see Davey, 1971a) in relation to the provenance of their samples, Beverley-Burton *et al.* (1977) conclude that only one species (*A. simplex*) is represented by Type I larvae in herring and salmon in the North Atlantic but that more than one population of this species may exist.

If *Anisakis* larva (I) of Japanese workers and *Anisakis* sp. larva (I) (= *A. simplex*) are identical, then this species is abundant in a great variety of fish and in squid in many localities (see section V).

The length range (23·0–31·7 mm) given for this larval type by Shiraki (1974) may be conservative; from juvenile herring in the North Sea the writers (unpublished observations) have recovered larvae 8·8 mm long and upwards, whilst Koyama *et al.* (1969) referred in a table to larvae up to 36·0 mm long.

2. Anisakis *larva (II)*

According to Shiraki (1974), Oshima (1972) and Koyama *et al.* (1969) this larval type is characterized by a short ventriculus with horizontal ventricular-intestinal junction, and a long, conical, tapering tail lacking a mucron. The head is similar to that of larva (I) but the boring tooth is said to be larger and directed anteriorly. For some morphometric data see Table 2.

Japanese workers regard this larval type as identical to *Anisakis* sp. larva (II) of Berland (1961). Some workers (e.g. Oshima, 1972) claim that it may be the larva of *A. physeteris* but proof is lacking; culture of the larva *in vitro* may be rewarding.

Larva (II) does not appear to be as common as larva (I) at least in Japanese waters (see section V). Berland's (1961) specimens of *Anisakis* sp. larva (II) came from *Lampris guttatus* in Oslo Fjord (Norway). According to Wheeler (1969), *L. guttatus* is uncommon in northern European waters; it seems to be most common in middle depths of the open ocean (especially south of Europe) and, perhaps significantly, feeds extensively on pelagic squid.

3. Anisakis *larva (III)*

This larval type is described by Shiraki (1974) as possessing a stout body, short ventriculus with horizontal junction, and a short, rounded tail without mucron (although Oshima, 1972, and Otsuru *et al.*, 1968, 1969, referred to a short, conical tail with a small mucron). The head is apparently similar to that of larva (II). Some morphometric data are given in Table 2.

Shiraki (1974) reported larva (III) in three teleost species in Japanese waters (see section V). The relationship of this larval type to an adult remains unknown; in-vitro culture does not appear to have been attempted.

4. Anisakis *larva (IV)*

According to Shiraki (1974) and Otsuru *et al.* (1969) this larval type is relatively small, has a short ventriculus with horizontal junction, and a short,

conical, pointed tail without mucron. The head is apparently similar to that of larva (II). For some morphometric data see Table 2.

Larva (IV) was recovered from *Theragra chalcogramma* in Japanese waters (Shiraki, 1974) and, like larva (III), nothing appears to be known of its relationship to an adult.

5. *Morphology of* Anisakis *larval types in cross section*

With the aim of identifying anisakid larvae in tissue sections of pathological specimens resected from the human alimentary tract, Japanese workers (e.g. Oshima, 1972; Shiraki, 1974) have provided illustrated descriptions of such features as the number of muscle cells in a quadrant of the body, number of intestinal cells, maximum body width and shape of the lateral chords as seen in transverse section at different levels. Discrepancies occur in the information on certain diagnostic features given by Oshima (1972) and by Shiraki (1974) for at least *Anisakis* larva (I) and larva (II), which tends to cast doubt on the merits of this system. Myers (1975) also expressed reservations about some of the criteria but suggested that further study may show morphology of the lateral chords in cross sections to be a valuable characteristic for diagnosis.

V. Geographical and Host Distribution of *Anisakis*

The geographical distribution of *Anisakis* and its distribution among host species is a complex and, as yet, poorly understood subject. As a genus *Anisakis* has a worldwide distribution occurring in all major oceans and seas but individual species are in some cases more restricted in distribution.

Anisakis simplex is probably the most cosmopolitan species, occurring worldwide in 23 cetacean and 11 pinniped species according to Davey (1971a). An additional three cetacean and one pinniped species were listed as hosts of *A. simplex* by Dailey and Brownell (1972). Records of *A. simplex* from marine mammals are most frequent in colder temperate and polar waters (Davey, 1971a). Within a given area there may be different populations of *A. simplex*, as suggested by Beverley-Burton *et al.* (1977) for the North Atlantic (see also section IV. E1).

Anisakis typica is listed from only eight cetacean species by Davey (1971a) who also noted that records of this species are restricted to warmer waters between 40°N and 36°S, a region where *A. simplex* is apparently rare. However, the taxonomic distinction between *A. simplex* and *A. typica* is problematical (see also section III and IV. B).

The relative importance of different cetacean species as hosts for *A. simplex* and *A. typica* is impossible to determine due to the lack of data on the population sizes of the hosts and the extent of their infection with *Anisakis*. It is likely that there are significant differences between geographical areas. Thus, Kagei and Kureha (1970) did not find *Anisakis* sp. in *Balaenoptera borealis* and *B. physalus* from the Antarctic Ocean although these species are known to be infected in other regions such as the North Pacific and North Atlantic (Klumov, 1963; Young, 1972).

Whilst pinnipeds have been widely recorded as hosts for *Anisakis simplex*

(Davey, 1971a) they are probably of limited importance in this role. In British waters, for example, *A. simplex* may be numerous in grey seals (*Halichoerus grypus*) but as only a small proportion mature successfully in this host (Young, 1972; Wootten, unpublished observations) it is thought that seals contribute little to the general abundance of the species.

Anisakis physeteris has been recorded from only four cetacean species according to Davey (1971a) and most records are from the sperm whale, *Physeter catodon*. The distribution of *A. physeteris* is worldwide (Davey, 1971a) paralleling that of its major final host.

Of the other species of *Anisakis* which may be regarded as valid, *A. insignis* is restricted to South American rivers, the habitat of its host the Amazon River Dolphin, *Inia geoffrensis* (Petter, 1972) and *A. schupakovi* is found only in the Caspian seal, *Pusa caspica*, from the Caspian Sea (Kurochkin, 1975).

Larval *Anisakis* have an extremely wide distribution in fish and squid both in terms of the range of host species and geographical distribution. The exact distribution of different *Anisakis* species is confused because no larva of *A. typica* has as yet been positively identified and also because many authors do not distinguish between "larval types".

Kagei (1970), Oshima (1972) and Shiraki (1974) listed the occurrence of *Anisakis* larvae by type in fish and squid in Japanese waters. *Anisakis* larva (I) is apparently the most abundant larval type, occurring in 123 fish species and in one squid species (*Todarodes pacificus*) (Oshima, 1972). High rates of infections with larva (I) were found in Pacific pollock (*Theragra chalcogramma*), cod (*Gadus morhua macrocephalus*), common mackerel (*Pneumatophorus japonicus japonicus*), herring (*Clupea pallasi*), horse mackerel (*Trachurus japonicus*), salmon (*Oncorhynchus* spp.) and squid (*Todarodes pacificus*).

Type (II) larvae are less abundant than type (I) larvae in Japanese waters, occurring in 25 fish species and two squid species (*T. pacificus* and *Doryteuthis bleekeri*) (Oshima, 1972).

In Japanese waters, type (III) larvae occur in the teleosts *Theragra chalcogramma, Gadus morhua* and *Synaphobranchus affinis*, whilst type (IV) larvae have been recovered apparently only from *T. chalcogramma* (Shiraki, 1974).

The occurrence of *Anisakis* larvae in fish and shellfish from Taiwan was listed by Yamaguchi *et al.* (1970) and Chen (1971), and in fish from the Philippines by Jueco *et al.* (1971). Hewitt and Hine (1972) listed 57 fish species as hosts for *Anisakis* larvae in New Zealand waters, including 10 species of elasmobranchs (in which, however, larvae were found only in the stomach); these authors suggested that the New Zealand larvae are probably *A. simplex*.

The Russian literature contains a number of records of anisakid larvae in fish from different oceans of the world. For example, Polyanski (1966) found *Anisakis* larvae in 28 species of fish from the Barents Sea; cod (*Gadus morhua*), saithe (*Pollachius virens*), redfish (*Sebastes marinus*), catfish (*Lycichthys denticulatus*) and bullhead (*Myoxocephalus scorpius*) were particularly heavily infected. Kurochkin and Leont'eva (1970) reported widespread infection with anisakid larvae of fish from the Indian and Pacific Oceans. Larval *Anisakis* were found in 19 species of fish from the Indian Ocean by Parukhin (1971),

members of the family Sauridae being especially heavily infected. Kurochkin (1972) referred to *Anisakis* sp. larvae in squid *Todarodes pacificus* in the Ussuriiskii Gulf (USSR).

Anisakis larvae are relatively common in some mesopelagic and bathypelagic fish, particularly myctophids (Collard, 1970; Noble and Collard, 1970). Larval *Anisakis* were widely but discontinuously distributed in mesopelagic fish but apparently absent from cold undiluted water masses (Noble and Collard, 1970). Larvae were also common in a number of macrourid species caught at depths of up to 4000 m from different areas (Noble, 1973). The means by which such deep-water benthic fish become infected with *Anisakis* is not clear. Collard (1970) suggested that mesopelagic myctophids acquire larvae in the epipelagic or surface waters during their diurnal vertical migrations by feeding on infected invertebrates. Myctophids could then in turn be preyed upon by bathypelagic species of fish and squid to which parasites might transfer. Collard did not believe that nematodes could survive such a transfer but subsequent research has shown that *Anisakis* larvae can in fact successfully transfer between fish (Smith, 1974). A downward transfer of *Anisakis* larvae from the epipelagic zone might account for at least part of the observed infections of deep-water benthic fish. On the other hand, invertebrates present at such depths, including euphausiids, which form part of the diet of macrourids (Noble, 1973), might act as intermediate hosts becoming infected by eggs and larvae descending through the water column from the epipelagic or mesopelagic zones where cetaceans are found.

Anisakis larvae have been recorded occasionally from purely freshwater fish. Thus Bassleer *et al.* (1973) found larvae in cultured trout in Belgium and Wootten and Smith (1975) reported larvae in trout from a British reservoir. In these cases trout had most probably become infected through being fed on untreated marine fish offal. Trout found infected in Ireland (Kane, 1966) and in Scotland (Kennedy, 1974) may have acquired larvae by similar means.

Anisakis larvae were recorded from seven species of fish in ponds and lakes in Dagestan (USSR) by Aligadzhiev and Altaev (1973) and in four species from the Tisa River basin by Ergens *et al.* (1975). It is not clear where these larvae originated but they may have been introduced with stocked fish fed on offal and subsequently spread to other species by predation and scavenging.

Except in the most general terms it is extremely difficult to determine the factors governing the distribution and abundance of *Anisakis* in a given geographical area. This is mainly due to the difficulties associated with adequate sampling of the wide range of hosts existing in any area in order to determine their relative importance in the life history of the parasite. Within the northern North Sea a number of studies have provided information on the biology and distribution of *Anisakis simplex* mainly in relation to the infection of food fish.

The major intermediate hosts of *A. simplex* in the northern North Sea are possibly euphausiids of the genus *Thysanoessa* (Smith, 1971). The highest prevalence of infection of *Thysanoessa* occurs within the central area of the northern North Sea with levels of up to 4% recorded at individual localities.

The average prevalence of infection of *Thysanoessa* spp. with *Anisakis* larvae is 0·3% which is a high figure given the abundance of these organisms and the quantity consumed by fish.

The high levels of euphausiid abundance and *Anisakis* infection in the central northern North Sea corresponds fairly well with the abundance of larvae in fish. The mean numbers of larval *A. simplex* in the musculature of whiting is much higher in this area than in waters off the Scottish East coast, the Shetlands and more southerly areas of the North Sea (Fig. 5) (Wootten and Waddell, 1977). The number of larvae in the musculature is positively correlated with the total number of larval *Anisakis* present in whiting. A similar geographical distribution of larvae has been observed in cod (Young, 1972; Wootten and Waddell, 1977), juvenile haddock and Norway Pout (Wootten, unpublished observations). Which fish species are of the greatest

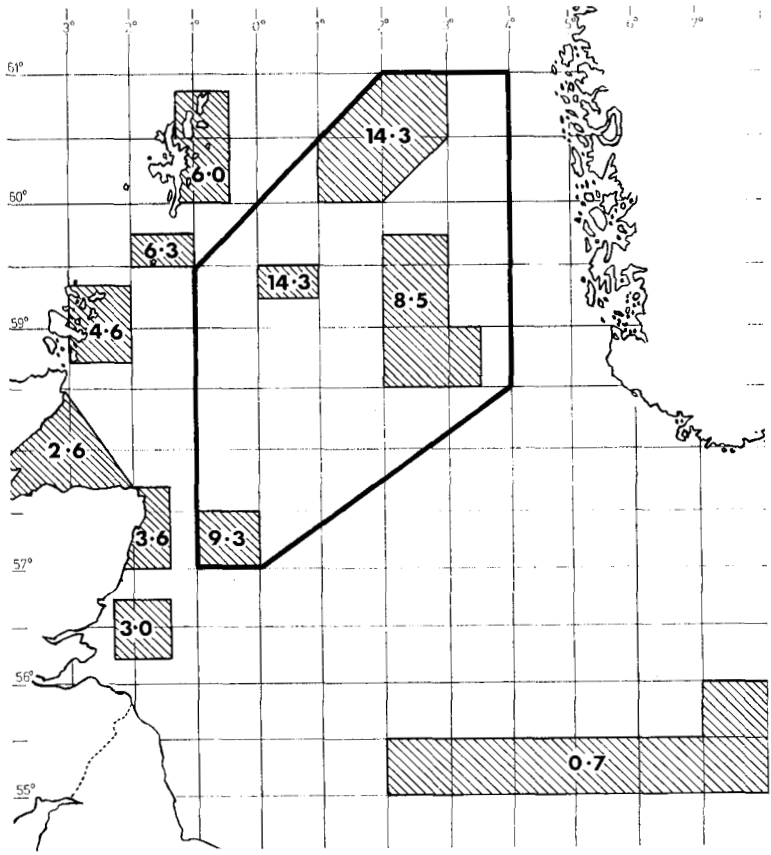

FIG. 5. Mean numbers of larval *Anisakis* in the musculature of whiting at various localities in the northern North Sea. Note that mean numbers of larvae are higher in the central area (delineated) than elsewhere.

importance in the transmission of *Anisakis* to the mammalian final hosts is uncertain. Although larger cod and whiting can be very heavily infected they may not be as significant in transmission as, for example, clupeoids (especially herring) or small gadoids which are an important source of food for the porpoise (*Phocaena phocaena*) (Rae, 1965) and possibly other cetaceans in the North Sea.

The abundance and distribution of cetaceans in the northern North Sea is little known. Porpoise, various dolphin species (including *Delphinus delphis, Tursiops truncatus, Lagenorhynchus albirostris* and *L. acutus*) and pilot whales (*Globicephala melaena*) are apparently the most abundant species (Evans, 1976). The relative importance of these cetaceans as hosts for *A. simplex* in the North Sea is unknown although small numbers of some species have been examined (Van Thiel, 1966; Young, 1972); among these, *P. phocaena, T. truncatus* and *L. albirostris* may contain large numbers of adult *A. simplex*. Cetaceans are found throughout the northern North Sea and there is no evidence that they are more abundant in the central area than in any other region. Seals are not significant hosts of *A. simplex* in the North Sea although a few adult parasites occur in young grey seals (*Halichoerus grypus*) on the Scottish East coast (Young, 1972; Wootten, unpublished observations). Such evidence as exists suggests that the abundance and distribution of the euphausiid hosts may be major factors determining the relative abundance of *A. simplex* in the northern North Sea.

The abundance of *Anisakis* may fluctuate quite dramatically with time. In the northern North Sea there appears to have been a large increase in the abundance of larval *A. simplex* in the musculature of whiting in recent years; in 1960–63 the prevalence of infection was less than 1·5% in over 9000 fish examined, but in 1971–74 the prevalence was 60% or more (Wootten and Waddell, 1977). There has also been a large increase in recent years in the infection of cod from Scottish waters with larval nematodes (Rae, 1963, 1972). Rae identified larvae as *Phocanema* but more recent work (Wootten and Waddell, 1977) has shown that larval *Anisakis* is very common in cod from some areas, notably the northern North Sea; it is probable that the increases in infection observed by Rae were due, at least in part, to *Anisakis*. Such apparent increases in abundance are probably due to changes in the populations of one or more of the host species but no obvious explanation is possible at the present time. There is no evidence of any increase in cetacean numbers in recent years. Possibly there has been a change in the abundance of euphausiids and/or the relative importance of euphausiids as a food item for fish. There have been large-scale changes in the plankton populations of the North Sea since 1950 (Cushing, 1975; Longhurst *et al.*, 1972) and, whilst some organisms such as the copepod *Pseudocalanus* have declined sharply in abundance, euphausiids appear to have at least held their own if not increased. As zooplankton is a major item in the diet of many fish species it is tempting to speculate that with a diminution in the availability of some organisms there has been a tendency for fish to feed more heavily on euphausiids and in this way to acquire heavier infections of larval *Anisakis*. This might lead to an increase in the over-all abundance of *Anisakis*. Many fish populations, especially

gadoids, have been at a high level in the northern North Sea since about 1960 (Longhurst *et al.*, 1972) and possibly this increase in "paratenic" host populations has also had an effect on the over-all abundance of the parasite.

VI. In-vitro Culture of *Anisakis* in relation to *In Vivo* Observations

Anisakis larvae from fish have been used by different workers to initiate cultures *in vitro* using various techniques and with varying degrees of success.

Grainger (1959) placed larvae from cod in an NA_2 HPO_4–citric acid buffer solution at pH 2·2 and 37°C. Addition of 1% pepsin assisted excapsulation but was not essential. The presence of pieces of fish for 24 h towards the beginning of the culture period was essential for the moult to preadult, which then occurred in three to five days; pepsin was not essential for the moult. Various morphological differences between larvae and preadults were noted. Attempts to rear adults were unsuccessful.

Khalil (1969) reported that larval *Anisakis* from herring moulted after four to five days at 36°C in a mixture of liver extract, horse serum and Medium 199. Size increase was seen from the seventh day but all died by 40 days in culture. Females developed better than males but the egg-laying stage was not reached.

Van Banning (1971) succeeded in culturing larvae from North Sea herring, mackerel and Norway haddock through to egg-laying adults in a digested liver extract (pH 2·0) with added beef blood. The most suitable temperature was between 34 and 37°C. At 10–11°C larvae did not moult but remained at this stage for up to at least 80 days. High mortality occurred at 38°C, and at higher temperatures up to 40°C development to adult was not completed. At 34°C the first moult occurred within four days. That pieces of cuticle were frequently found when the medium was renewed suggested that there are two or three moults before the worm matures (but this aspect is not elaborated). The preadult stage varied in duration from at least 26 days to a maximum of 98 days. The phase to full maturation was short at about seven days. Male worms measured 3·5–7·0 cm, females 4·5–15·0 cm; they were subsequently identified as *A. simplex* by Pippy and Van Banning (1975). Mature males were placed in tubes with just maturing females; fertilization took place and viable eggs were obtained which hatched satisfactorily. Grabda (1976) cultured larvae from Baltic herring through to the adult stage using a modification of Van Banning's technique with essentially similar results; again the adults were identified as *A. simplex.*

Schulz (1974) cultured larval *Anisakis* from herring to preadult in a medium comprising CEE, liver extract, protein hydrolysate and pig serum. At 39°C in a gas phase of 10% CO_2 in air, moulting was first seen on the third day; on average, 50% survived for 18 days but no further development was seen.

Sommerville and Davey (1976) have shown that development of third-stage *Anisakis* larvae (from barracouta, *Leionura atun*) and the moult to preadult can take place in a far simpler medium than any used by the above workers; this paper contains the essential results given by Sommerville (1974) and

Sommerville and Buzzell (1974). These authors found that larvae can form a new cuticle and moult to preadult *in vitro* at 37°C in about three to five days but that relative success depended on certain conditions. In a culture medium of Krebs–Ringer balanced salt solution (BSS) under 5% CO_2 in air, relatively few moulted and survival was poor. More larvae moulted and survival was enhanced in Medium 199 even if the gas phase was air, but the presence of 5% CO_2 led to a quicker moult. The benefits of high CO_2 concentrations with both BSS and Medium 199 accrued only if this gas was present during the first 40 h of incubation. Worms were harmed at temperatures near 40°C. The pharynx of moulted worms (fourth stage) usually contracted vigorously whilst that of third stage larvae was invariably immobile. These observations and studies on uptake of serum conjugated with rhodamine suggested that worms do not feed until after they have moulted, a conclusion also reached by Yasuraoka *et al.* (1967) and Koyama *et al.* (1967). That external physico-chemical stimuli may trigger internal regulatory mechanisms leading to development of *Anisakis in vitro* is related by Sommerville and Davey to similar observations on strongyloid nematodes (e.g. *Haemonchus*).

There is always the possibility of abnormality in growth and development during in-vitro culture of a parasite. However, in several respects the above results obtained for *Anisakis* fit fairly well with observations *in vivo* on the course of events in experimentally infected laboratory animals, especially that concerning the moult to preadult. For example, Gibson (1970) studied development of *Anisakis* larvae from herring in rats. He described and illustrated morphological changes in the head, ventriculus, gut wall and cuticle that accompanied the moult from larva to preadult. Larvae showed no signs of sexual development but after three to four days in the rat they moulted and some showed a developing vulva, vagina and coiled uteri; development of a male system was not seen. Some preadults, particularly those which had spent 11 days or more in rats, showed cuticular separation especially at the anterior end which suggested that another moult was about to take place. Gibson's observations *in vivo* are in general agreement with those obtained *in vitro* by other workers for the moult to preadult.

Apart from the observations recorded by Van Banning (1971) and Grabda (1976) during their culture work *in vitro*, little information is available on the preadult to adult phase of development. In-vivo studies with laboratory animals offer little insight into this aspect and it is unfortunate that investigation of the natural final hosts is fraught with practical difficulties. Marine mammals are large animals, difficult and expensive to maintain in captivity; captive animals have commercial value as a public spectacle and this and other factors, including aesthetic ones, may preclude experiments on them. Marine mammals shot in the wild or found dead or dying have been used for study, but post-mortem changes may lead to difficulties of interpretation. Therefore, the following conclusions reached by different workers from observations on wild-caught animals should be viewed with caution.

Kagei *et al.* (1967), according to Oshima (1972), observed *A. simplex* or *A. typica* in blue–white dolphins. During the moult to the fourth stage (preadult) these authors noted loss of the boring tooth and mucron and

acquisition of distinct transverse striations to the cuticle more than 17 μm apart. Worms more than 30 mm long had moulted to the fifth (adult) stage, showing an open vagina in females and paired spicules and caudal papillae in males; the cuticular striations were 23–42 μm apart.

Kikuchi *et al.* (1967) studied *Anisakis* sp. in dolphins (*Tursiops gilli*), and suggested that third-stage larvae invade the gastric mucosa and submucosa and grow to a length of 20–27 mm before moulting. The preadults are then said to return to the stomach lumen and to attach themselves to its wall; after further development and at a length of 28–35 mm they are said to undergo another moult to achieve the adult stage. Whilst the observation of two moults in the final host may be correct, there are reasons to doubt deep tissue penetration and growth to 20–27 mm within tissues before the moult to preadult. The work of Vik (1964) and Young and Lowe (1969) suggests that development and moulting occur during superficial attachment to the alimentary tract in the final host. Furthermore, Gibson (1970) observed a preadult only 13 mm long in experimentally infected rats.

A wide range in length has been recorded not only for stages recovered from wild crustaceans, fish and squid but also for adult males and females obtained from final hosts and *in vitro*; it may be unrealistic, therefore, to expect any correlation between length and the timing of a particular moult.

VII. Physiology and Biochemistry of *Anisakis*

A wealth of detailed information is available on the nutrition, digestion and general metabolism of nematodes in general, as is evident from the reviews of Brand (1966), Bryant (1975) and Lee and Atkinson (1976), and of the various contributors to chapters in Florkin and Scheer (1969). However, the available information on these aspects for *Anisakis* is fragmentary and often relates only to larvae removed from fish.

Metabolic studies by Hamajima *et al.* (1969) on *Anisakis* sp. larvae indicated that the Embden-Meyerhof pathway might act as an energy supply from several carbohydrates. Krebs' tricarboxylic acid cycle oxidation and a succinoxidase system also appeared to be present. Kawai (1968) separated long-chain fatty acids from various organs of larvae by gas chromatography.

Anisakis larval emulsions were examined for lactate dehydrogenase isozymes by Nagase (1968a) using cellulose acetate chromatography; the two bands that appeared were identified as LDH–1 and LDH–2 known from human sera.

The amylase activity in extracts of *Anisakis* larvae reported by Taniguchi (1967) was active over a pH range of 4·0 to 9·0, with two maxima (at pH 4·0 and at pH 7·0). With regard to isozymes, electrophoretic and zymogram analyses revealed six distinct protein fractions showing amolytic activity.

Ruitenberg (1970, 1972) and Ruitenberg and Loendersloot (1971a,b) reported on enzyme histochemical tests on *Anisakis* sp. larvae taken from herring; various organs and tissues were studied, in some cases with the larvae penetrated (experimentally) into rabbit stomachs. The main results are summarized below.

The cuticle showed weak activity by only three enzymes (5-nucleotidase, adenosine triphosphatase and phosphorylase). In the musculature, moderate to strong activity was shown by various oxidative enzymes. Alkaline phosphatase was absent from the brush border of the intestinal tract, but here other phosphatases (acid phosphatase, 5-nucleotidase and adenosine triphosphatase) showed moderate to strong activity. Leucine aminopeptidase activity was also strong in the brush border. Intracellularly, the epithelial cells of the intestinal tract showed strong PAS activity shown to be glycogen; of the phosphatases, only adenosine triphosphatase showed some activity. Polyacrylamide gel electrophoresis showed only one band of acid phosphatase activity, i.e. isozymes were absent.

The "excretory organ" of larvae showed slight to strong activity by 18 enzymes. Non-specific esterase activity was especially strong in the lateral part of the organ, with the central area showing moderate activity. Some leucine aminopeptidase activity was also present.

Protein-rich substances, possibly histolytic enzymes, may be contained in the membrane-bound secretory granules noted by Lee *et al.* (1973) in their ultrastructural study of the "excretory system" of *Anisakis* larvae; these granules occupied much of the gland. Mueller (1927) also referred to secretory granules in the ventral gland of the "excretory system" of adult *Anisakis simplex*. The various roles that have been ascribed to the so-called "excretory system" of nematodes in general include excretion, osmoregulation, larval exsheathment, penetration of host tissues and extracorporeal digestion (see, for example, Wright and Newall, 1976); these matters are controversial and the position regarding *Anisakis* in particular is not clear.

Little is known of the tissue penetration mechanism(s) of *Anisakis* in either cold-blooded or warm-blooded hosts. Matthews (1977) examined enzymic activity in larval *Anisakis* (from herring) using an azocoll dye release technique. Secretions showing azocoll positive activity were released at temperatures above 10°C but the optimum temperature both for release and for activity against the substrate appeared to be about 37°C, the body temperature of marine mammalian final hosts. This result is somewhat anomalous in that more extensive larval migration appears to occur in fish than in marine mammals; further work is in progress.

Okuno (1968, 1969) reported 18 amino acids in hydrolysed tissues of *Anisakis* sp. larvae. Proline, alanine, glycine and glutamic acid were most common, whilst tryptophan, lysine, histidine, arginine and cystine were found in very small amounts. Oishi *et al.* (1972a) reported 17 amino acids in larval *Anisakis* from Alaska pollack and recorded differences in the abundance of certain of these depending on the locality of host capture (Okhotsk Sea and Pacific Ocean). Moreover, larvae from pollack contained three or six times more aspartic and glutamic acid than larvae from mackerel, but less tyrosine, histidine and arginine.

The amino-acid composition of the cuticle and of the TCA supernatant and precipitated fractions of the reproductive organs and perienteric fluid of adult male and female *Anisakis physeteris* (from sperm whales) was reported by Viglierchio and Görtz (1972a). The amino-acid nitrogen of the cuticle

accounted for about 96% of the total nitrogen; over 50% of the cuticle protein hydrolysate consisted of proline, glycine and arginine. In reproductive organ fractions, lysine, glutamic acid, glycine, valine, leucine and histidine accounted for about 62% of total nitrogen. Interesting differences were recorded in the amounts of certain amino acids present in corresponding tissues of male and female worms, and in different tissue fractions from worms of the same sex: there was more serine in female than male cuticle; there was more proline in the protein of female than male reproductive organs; methionine was present in cuticular protein and in reproductive organ protein and non-protein fractions but absent from perienteric fluid; cystine was present in cuticular protein but not in other fractions. From an increase in the ammonia content of stored untreated perienteric fluid, Viglierchio and Görtz (1972a) suggested tentatively that ornithine cycle and amino-acid oxidase activity may operate *in vivo*.

Suzuki *et al.* (1974) reported on the haemoglobin extracted from the perienteric fluid of larval *Anisakis* as an antigen in immunodiagnosis but no information on its spectral or other characteristics was given. Viglierchio and Görtz (1972b) found that a partially purified haemoglobin from the perienteric fluid of adult *Anisakis physeteris* differed in spectral and reactive properties from the haemoglobin and myoglobin of both the sperm whale host and the fin whale. Deoxygenation with sodium dithionite of the worm haemoglobin and the cetacean haemoglobins and myoglobins took less than one minute. Oxidation with potassium ferricyanide of the cetacean pigments was also rapid, but t_{50} values for oxidation of worm haemoglobin were high: 450 s for pigment from male worms, 660 s for that from females. Another sex difference in worms was found in the iron and copper content of the haemoglobin—respectively, 3·90 and 0·195 mg 100 ml^{-1} for males, and 3·00 and 0·180 mg 100 ml^{-1} for females. *Anisakis* haemoglobin may function more in the transport of oxygen than in its storage but further work is needed.

Williams and Jones (1976) recounted experiments at Aberdeen University the unpublished results of which showed that larval *Anisakis* can survive for at least eight days at 12°C in dilutions of sea water from 0% (distilled water) to 100% (normal sea water). Various other approaches including weight loss/gain in different dilutions and a silver staining technique suggested that the cuticle is relatively impermeable; this might explain the survival of larval *Anisakis* in a wide range of hosts but comparable studies on preadults and adults have not been conducted.

VIII. Pathology of Anisakiasis

The following account of pathology associated with *Anisakis* infection deals with various developmental stages of the nematode in various animals, be they "natural", "accidental" or experimentally infected hosts. For convenience the animal groups are treated in ascending phylogenetic sequence. Squid (molluscs) are omitted because no relevant reports appear to be available. The pathology of anisakiasis of man is dealt with in section IX. B.

A. CRUSTACEANS

No direct observations of pathology in crustaceans have been reported. It is interesting to note, however, that whilst multiple infection of euphausiids with freshly-hatched second-stage larvae of *Anisakis* was achieved experimentally by Oshima (1972) (see also section IV. C), all reports of natural infections in crustaceans refer to only one larva per infected host (see section IV. D). It is tempting to speculate that multiple infection eventually kills hosts, and that only hosts infected with a single larva are able to survive. On the other hand, it is possible that one or more mechanisms operate to control the parasite number during the course of larval development; Halvorsen and Williams (1968) discussed some of the literature on this phenomenon in relation to their observations on *Gyrocotyle* in *Chimaera*. Clearly, comparative studies on the histology of infected and uninfected crustacean hosts and an experimental approach are required.

B. FISH

The effects of *Anisakis* larvae on fish are not clear. Several workers have suggested that larvae can adversely affect the condition of infected fish but many of these studies lack firm evidence and seem rather subjective in nature. The literature on the effect of larval *Anisakis* on fish has been reviewed previously by Margolis (1970) and Cheng (1976).

Larval *Anisakis* are found encapsulated throughout the viscera and musculature of fish although the actual distribution within the tissues varies between fish species. For example, in cod from Scottish waters, less than 12% of the total number of *Anisakis* larvae present are found in the musculature whereas in the whiting over 50% occur in this site (Wootten and Waddell, 1977). Within the viscera, larvae are found mainly on the surface of the liver or among the mesenteries, especially those surrounding the gut. Polyanski (1966) gave data on the occurrence of larval *Anisakis* in these sites in 28 species of fish from the Barents Sea. Larvae may be found free in the gut lumen of the host or may be seen at different stages of penetration through the gut mucosa (Sluiters, 1974); presumably such larvae have been only recently acquired by ingestion of infected intermediate hosts.

Encapsulated larvae are normally tightly coiled in a spiral (Fig. 6). The process of encapsulation and the nature of the capsule in the liver of fish were studied by Kahl (1938), Mikhailova *et al.* (1964) and Prusevich (1964). The capsule is of host connective tissue origin and three-layered. The inner layer is thin and consists of damaged cellular elements; this layer originally surrounds each coil of the larva. The middle layer is made up of a large proportion of partly degenerated fibroblastic elements, whilst the outer layer consists of loose connective tissue and may be slightly vascularized. The capsule is continually eroded from within and built up on the outside. The thickness of the capsule depends on the quality of host connective tissue nearby and on the age of the capsule. Older capsules in which the nematode has died are reduced

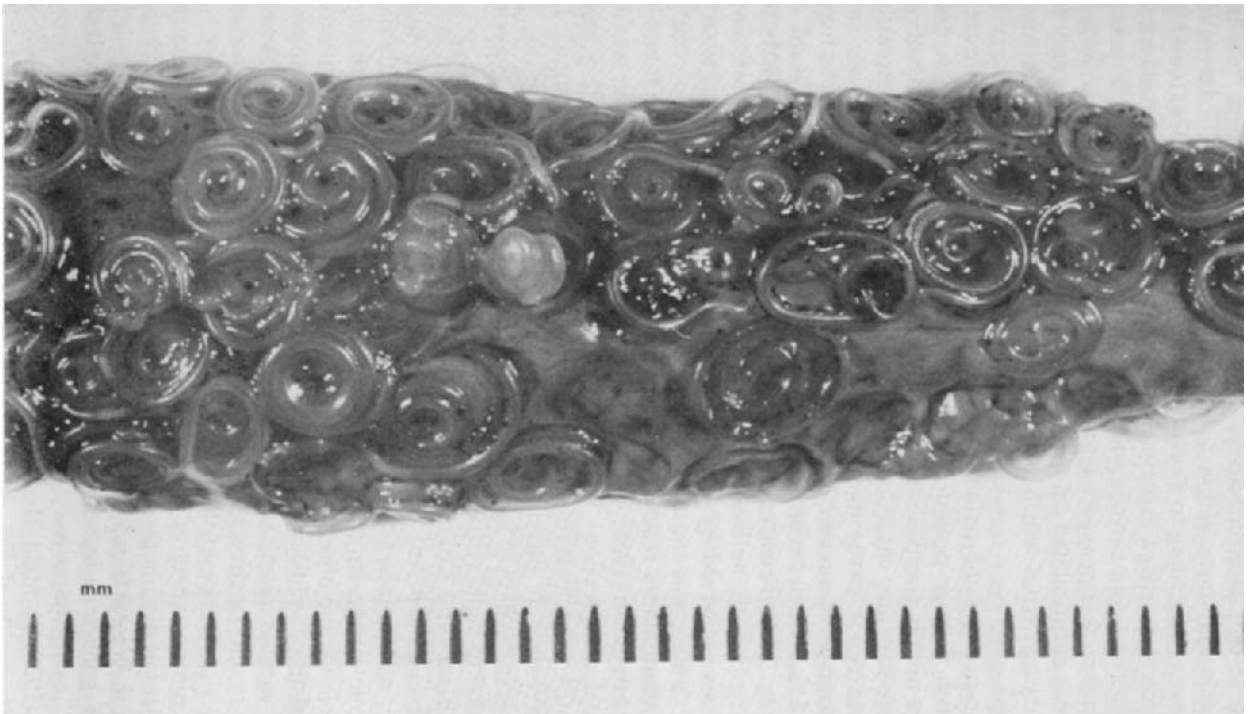

FIG. 6. Portion of liver from a gadoid fish heavily infected with encapsulated *Anisakis* larvae.

in size and may become calcified. Kahl (1938) compared the structure of capsules from different sites in several fish species and, in all cases, found it to be similar to that described above.

Encapsulation starts within a few hours of the arrival of larvae in the visceral cavity according to Prusevich (1964), and Smith (1974) found larvae in the visceral cavity of experimentally infected whiting and haddock to be surrounded by a thin capsule within 24 hours of infection. It is of interest to note, however, that in experimentally infected trout in fresh water, larvae had not become encapsulated up to 24 days after infection (Wootten and Smith, 1975) although trout which had been infected for some months were encapsulated.

Most reports of the pathogenic effect of larval *Anisakis* in fish are concerned with damage caused by the larvae to the liver of the host. *Anisakis* larvae are usually encapsulated just under the connective tissue capsule of the liver where they may cause local damage, probably mainly by compression of the tissues. Some larvae may penetrate more deeply into the liver causing greater damage. A number of authors have reported a reduction in liver size in several fish species with heavy infections of larval *Anisakis* (Kahl, 1938; Akhmerov, 1951; Petrushevski and Kogteva, 1954; Remotti, 1933; Brian, 1958). Atrophy of the liver of hake in heavy but superficial infections of *Anisakis* was observed by Remotti (1933) but Guiart (1938) suggested that this liver damage was caused by toxins secreted by the worm. No evidence of general inflammatory reaction or degenerative liver changes was found in the same host by Brian (1958) who concluded that atrophy of the liver was due to mechanical compression. When larvae died Brian suggested they released "thanatotoxins" which caused local liver damage and that undamaged liver tissue increased its physiological activity, thus accounting for the slight differences in the flesh of hake with infected and uninfected livers. No evidence is presented to support these latter views.

Petrushevski and Kogteva (1954) reported a fall in the condition factor of *Myoxocephalus scorpius* with increasing liver infections of larval *Anisakis*. However, as Margolis (1970) pointed out, these authors apparently did not take account of age, length or spawning condition of the hosts which might be responsible for the observed differences in condition. The same authors also compared the condition factor of cod and *M. scorpius* in relation to their infection with larval anisakids (including *Anisakis*) but made no allowance for the identity and location of larvae in different hosts. Both these factors could affect the significance of the observed infections (Margolis, 1970).

No significant effect of larval *Anisakis* infection on the condition factor, average length distribution or gonad weight of anchovy (*Engraulis capensis*) from South West Africa was recorded by Hennig (1974); the "underdevelopment" of gonads in some fish was thought to be due to "mechanical suppression" by the parasite.

Kahl (1938) thought that severe infections with larval *Anisakis* could impair the health of fish. In some cod, Kahl found large tubercles in the stomach wall containing many degenerating and live larvae. Heavy infections in the body cavity of redfish (*Sebastes marinus*) caused visceral adhesions.

Anisakis larvae in ulcerous cavities in the stomach wall of severely emaciated *Ophiodon elongatus* were recorded by Arai (1969) who considered that the larvae may have been responsible for the condition of the fish.

Bishop and Margolis (1955) found that the frequency distributions of *Anisakis* sp. larvae in Pacific herring were skewed. Using this data, preliminary calculations by Lester (1977) showed that truncation at the higher end of the frequency distributions did not affect the parameter N, the total numbers of fish expected to be present according to the theoretical distributions involved; this suggested that little or no mortality of herring was associated with larval *Anisakis* infection.

There is little conclusive evidence that larval *Anisakis* are serious pathogens of fish; there is a need for carefully controlled experimental infections to be carried out to determine with certainty the effect of larvae on fish.

C. AMPHIBIANS

According to Oishi *et al.* (1969), larval *Anisakis* were administered to bullfrogs by Hirao and Yamaguchi (1964). Larvae quickly migrated into the abdominal cavity but haemorrhages were observed in the stomach wall two days after infection. After five days larvae were becoming encysted, but most were still alive 30 days after infection and were capable of reinfecting other bullfrogs.

D. REPTILES

Farmed green turtles (*Chelonia mydas*) may become infected by "worms" acquired in fish feeds (Clayton, 1975). The growth of infected turtles may be "stunted" and Clayton pointed out the danger of worms migrating into the flesh. Given the ability of larval *Anisakis* to transfer between hosts (Smith, 1974; Wootten and Smith, 1975) and their common occurrence in fish, it is quite likely that the worms referred to by Clayton were larval anisakids, possibly including *Anisakis*.

E. BIRDS

There are surprisingly few records of *Anisakis* in piscivorous birds in view of its abundance in marine fish. The paucity of records may only reflect a lack of parasitological studies on piscivorous birds rather than a real scarcity of *Anisakis* in these hosts. Kreis (1958) records an immature female *Anisakis* sp. larva in the oesophagus of a fulmar (*Fulmarus glacialis*), whilst Bakke and Baruš (1975) found *A. simplex* larvae in the oesophagus and ventriculus of common gulls (*Larus canus*) from Norway. In the North-West Pacific Ocean, *Anisakis* sp. larvae were recorded in *Larus argentatus* and *Lunda cirrhata* by Belogurov *et al.* (1968) and in *Larus crassirostris, Oceanodroma monorchis* and *Phalacrocorax ussuriensis* by Alekseev and Smetanina (1968).

On the east coast of Britain, ulceration of the proventriculus of fulmars caused by *Anisakis* sp. larvae and preadults (probably *A. simplex*) was described by Riley (1972). Clusters of nematodes were found at the centre of

ulcerous swellings, with each worm lying at the centre of a necrotic area. Histolysis was present around the anterior ends of the worms. The nematodes had penetrated into the submucosa and affected the muscularis. The proventricular glands were extensively vacuolated in the region of penetration. Riley (1972) suggested that as only a small proportion of the very large proventriculus of the fulmar was destroyed by the ulcers it was unlikely that the functioning of the organ would be seriously affected. Secondary infection of the ulcers may occur.

F. MARINE MAMMALS

One of the earliest records of pathology in marine mammals caused by *Anisakis* was apparently that of Murie (1868) who described ulceration of the fundic stomach of the walrus caused by "*Ascaris bicolor*" (= *A. simplex*) which he believed led to the death of the host. Subsequently a number of authors have described ulceration of the stomach of pinnipeds and cetaceans by *Anisakis* spp.

A detailed description of lesions from the stomach of grey seals (*Halichoerus grypus*) and porpoises (*Phocaena phocaena*) was given by Young and Lowe (1969). These authors found groups of 50–100 larval and "immature" *Anisakis* associated with inflammatory areas 1–6 cm in diameter in the fundic stomach of the grey seal. Such aggregations of *Anisakis* were found only in juvenile grey seals. The anterior ends of the nematodes were embedded in the mucosa and submucosa at the centre of the lesions and were surrounded by fibrin and inflammatory cells. The lips of the nematodes were surrounded by an amorphous eosinophilic substance and fibrin layer which was in turn enclosed by an area of massive infiltration. In some parts of the lesions inflammatory areas surrounding cast nematode cuticles were found in the submucosa. A large amount of granulation tissue was found around each inflammatory focus in the mucosa and produced a raised inflammatory area.

In the porpoise Young and Lowe (1969) found lesions mainly in the first stomach (Figs 7 and 8) but they also occurred in the second and third stomachs. In most lesions only "immature" *Anisakis* were found but in some cases adult *A. simplex* also occurred. The histology of the lesions from porpoise resembled that in grey seals although there were many more inflammatory areas with cast cuticles in the former host. These areas were often calcified. Large masses of granulation tissue were formed around the lesions which greatly thickened the submucosa and, in some cases, also the epithelial and muscular layers of the stomach wall.

Similar lesions were described by Vik (1964) in the stomach of porpoise caused by clusters of *Anisakis* larvae. Cowan (1967) reported a severe inflammatory response in the peptic stomach of pilot whales (*Globicephala melaena*) to infection by groups of *Anisakis*. Inflammatory reactions and ulceration caused by *Anisakis* were found in the bottlenose dolphin (*Tursiops gilli*), pacific white-sided dolphin (*Lagenorhynchus obliquidens*), Risso's dolphin (*Grampus griseus*) and rough-toothed dolphin (*Steno bredanensis*) by Kikuchi *et al.* (1967) and in the blue–white dolphin (*Stenella caeruleoalba*),

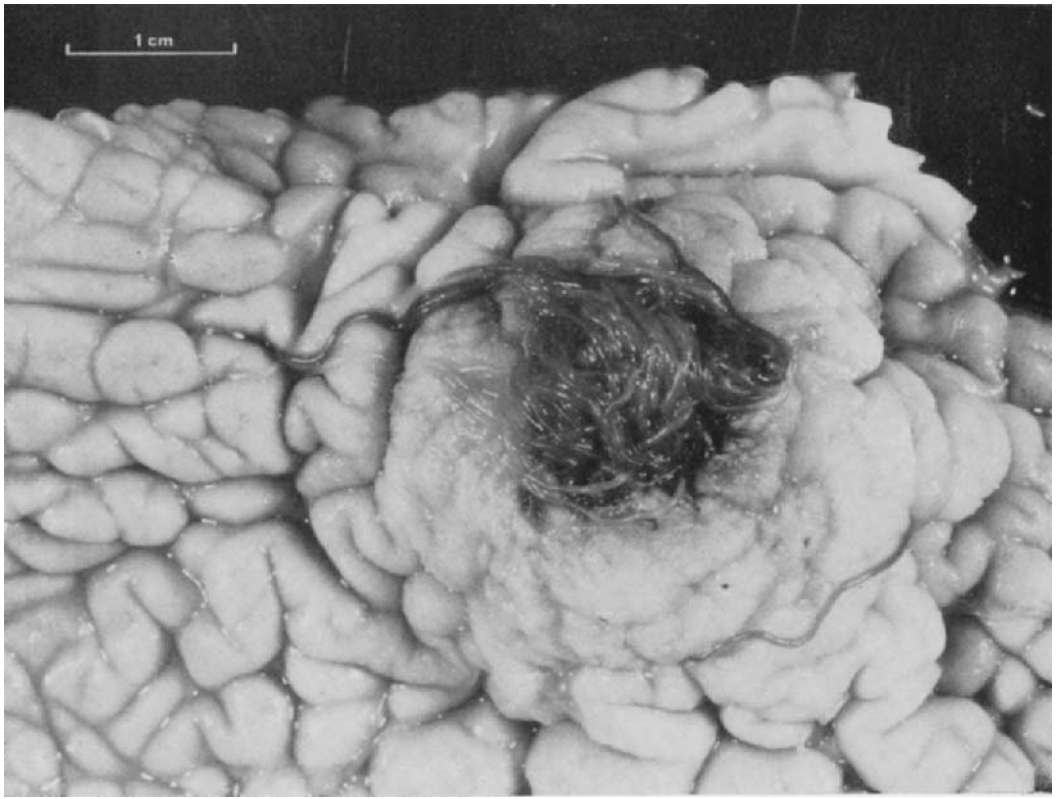

FIG. 7. First stomach of porpoise (*Phocaena phocaena*): lesion containing a cluster of larval and preadult *Anisakis*.

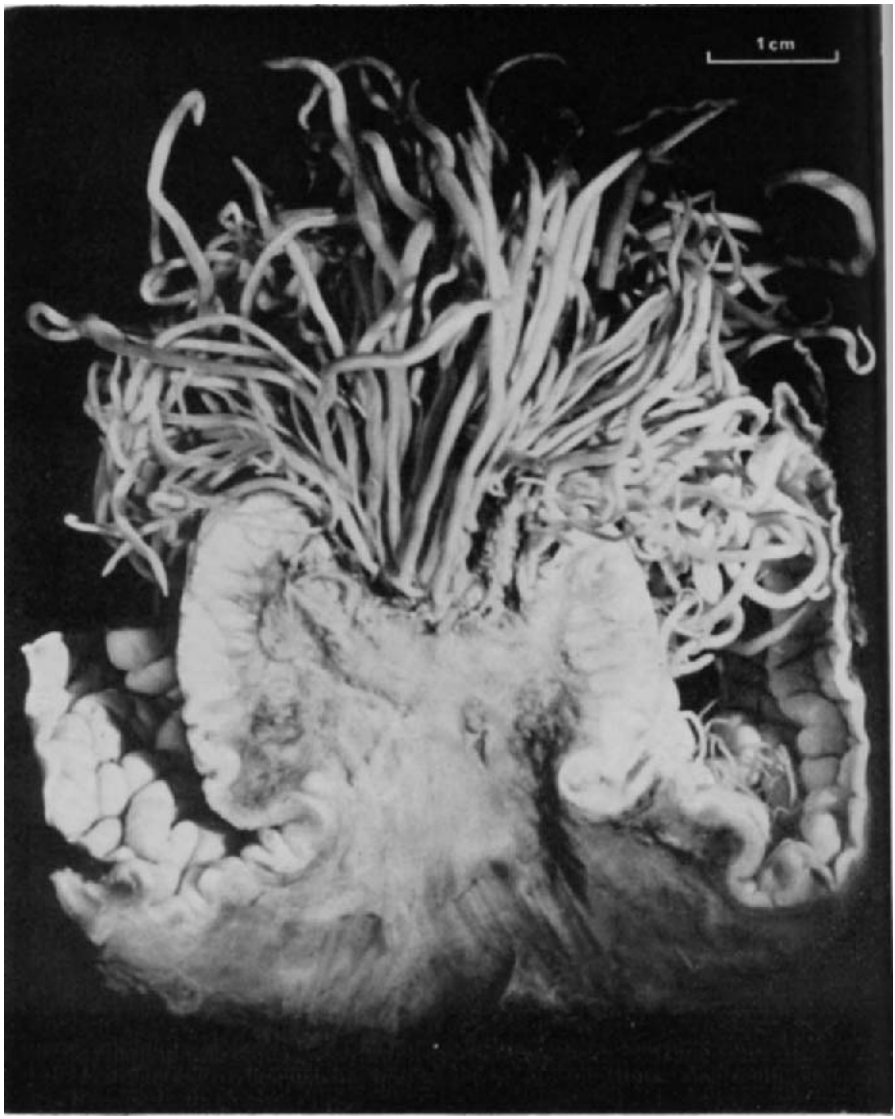

FIG. 8. First stomach of porpoise (*Phocaena phocaena*): bisected lesion, more severe than that shown in Fig. 7, containing a large cluster of "immature" and adult *Anisakis*. (Courtesy D. Lowe.)

Dall's porpoise (*Phocaenoides dalli*) and *Phocaena phocaena* by Kagei *et al.* (1967). Testi *et al.* (1971) described ulceration caused by *Anisakis* sp. in a common dolphin (*Delphinus delphis*) whilst Mikagi *et al.* (1971) suggested that gastric ulcers in the California sea-lion (*Zalophus californianus*) were due to *Anisakis* sp.

The severity of gastric lesions in marine mammals is presumably due to the mechanical irritation of large numbers of *Anisakis* larvae penetrating the stomach wall at one point. Toxic secretions from the parasites may also have some effect on the stomach wall. The stimulus causing the aggregation of *Anisakis* larvae at one point is unknown but it may be that the initial attachment of only a few parasites will cause a lesion which then provides a more suitable site for other individuals.

According to Riley (1972), Hoeppli (1933) found that tissues around the anterior end of *Anisakis* sp. in the stomach wall of the walrus were necrotic and suggested that they were liquified by secretions from the oesophageal glands of the nematodes. In addition, Hsü (1933) described a positive correlation between the size of the dorsal oesophageal gland and extracorporeal digestion in some ascaridoids, including *Anisakis*. The glandular secretions of a large cluster of *Anisakis* sp. may produce a partially digested mass of tissue upon which the nematodes may feed (Riley, 1972). Repeated infections of larval *Anisakis* or presensitization in experimentally infected mammals may cause an increased host response (see also section X. B) and it is possible that such an effect occurs in naturally infected marine mammals.

The cast cuticles observed in lesions by some authors may be those of larvae which have invaded the mucosa before moulting to the fourth stage. Adult *Anisakis* are normally only superficially attached to the stomach wall (Kikuchi *et al.*, 1967; Young and Lowe, 1969) and thus do not cause ulceration. The location of ulcers within the stomach of marine mammals was suggested by Young and Lowe (1969) to reflect the area where larvae are activated after ingestion by the host. Ronald (1960, 1962, 1963) suggested that in the related genus *Terranova* (= *Phocanema*) the stimulus causing movement of larvae ingested with fish is the high temperature of mammalian stomach fluids. Larval anisakids may penetrate into the gut wall in the first area where food is macerated regardless of any other stimuli. This area is the fundic stomach of pinnipeds and the first stomach of cetaceans. Lesions reported to be caused by *Anisakis* and other anisakid nematodes predominate in these regions.

Blessing and Peitz (1970) described ulcers caused by *Anisakis* in the Atlantic bottlenosed dolphin (*Tursiops truncatus*) and *Zalophus* sp. and suggested that ulcers healing spontaneously may produce stenoses in the pyloric region which contribute to the death of the host. In a similar case, Gembardt *et al.* (1971) reported that dilatation of the stomach wall in a sea-lion (*Mirounga leonina*) caused by a pyloric stenosis, due to inflammation and ulceration resulting from an *Anisakis* infection, led to chronic congestion in the abdominal venous system and eventually to the death of the animal by circulatory decompensation. Anisakid nematodes in the stomach wall of the California sea-lion (*Zalophus californianus*) may on occasion perforate the gut wall and be found in the peritoneal cavity (Griner, 1974).

Gastric nematodes of marine mammals kept in captivity have been successfully removed by anthelmintic drugs. Ridgway (1972) recommended thiabendazole at a dose rate of 45 mg kg^{-1} body-weight repeated after three months and then at six-month intervals. In severe cases, an initial dose of 70 mg kg^{-1}.

can be administered followed by a further dose of 45 mg kg^{-1} in 1–3 weeks. Sweeney and Ridgway (1975) suggested a dose rate of 73 mg kg^{-1} for thiabendazole. The same authors reported moderate effectiveness of piperazine at a dosage of 55 mg kg^{-1} and good effectiveness of levamisole at 8–11 mg kg^{-1}. If reinfection of captive mammals is prevented by the use of a frozen fish diet, nematodes are lost and ulcers heal within six months (Griner, 1974).

G. OTHER MAMMALS (EXCLUDING MAN)

Various mammals have been used as experimental animals to elucidate the pathology of *Anisakis*, particularly in relation to the problem of the response in sensitized hosts (see also section X. B). Larvae administered orally to rabbits mainly penetrated the stomach and caused lesions similar to those recorded from man (see section IX. B). Ruitenberg *et al.* (1971) found that where a number of larvae had invaded the gastric mucosa at a single point an ulcer surrounded by haemorrhages was present. Three days after infection, larvae were surrounded by necrotic tissue with granulocytes including many eosinophils. After five days, plasma cells were present and fibroblasts appeared at the periphery of the inflamed area. By ten days the degenerating larvae were surrounded by extensive granulation tissue outside which were large numbers of fibroblasts. Larval remnants eventually became surrounded by young connective tissue which, in turn, was enclosed by extensive mononuclear infiltration. After some months the larval remnants showed signs of calcification. Normally, the host reaction around a single larva penetrating the stomach wall was slight but such infections could produce a severe reaction.

Japanese workers found that sensitized rabbits showed a much stronger reaction to invading *Anisakis* larvae, including the formation of eosinophilic granulomata, than did non-sensitized animals in which the "foreign-body" type of reaction was observed (Oishi *et al.*, 1969). The reaction of experimental hosts to invading larvae was strong in rabbits, less so in dogs and only slight in cats. In sensitized dogs, in contrast to rabbits, infiltration of neutrophils occurred but there was no formation of granulomata, whilst in cats only slight neutrophil infiltration occurred. In dogs, the ratio of larvae invading the stomach and intestine was 1:1 but in rabbits the ratio was 17·8:1 (Oishi *et al.*, 1969).

In smaller mammals, such as rats and guinea-pigs, larvae mainly attack the stomach wall and a large proportion pass right through the wall and migrate amongst the viscera. Larvae are capable of migrating through the stomach wall of rats within 24 h of infection (Oishi *et al.*, 1969). Larvae penetrating the stomach wall of rats were surrounded by a thick layer of eosinophils and lymphocytes after 24 h; large numbers of red blood cells were present in the submucosa (Young and Lowe, 1969). Four days after infection larvae and cast sheaths were surrounded by numerous eosinophils and lymphocytes in which macrophages and fibroblasts were scattered. The submucosa showed a general eosinophilic infiltration and numerous lymphocytes. Reinfected rats showed similar cellular responses but these were both more rapid and more intensive. A more intense reaction to invading larvae in sensitized rats was

also seen by Japanese workers (Oishi *et al.*, 1969). Larvae do not survive for much more than seven days after they have penetrated the gut wall (Young and Lowe, 1969). According to Oishi *et al.* (1969), necrotizing larvae were found in localized granulomata in the body cavity, but after 40 days no traces of larvae could be seen. Okumura (1967) found that *Anisakis* larvae from different fish hosts showed different patterns of penetration of the alimentary tract of rats and survived for varying periods of time in this host.

Myers (1963) found that larvae fed to guinea-pigs penetrated the stomach wall and migrated around the viscera where they could be found alive for up to five days but had disappeared after six days. Macroscopically, inflammation and haemorrhage could be seen at the point of invasion of the gastric wall and infiltration of leucocytes and macrophages occurred around larvae.

The pathology of *Anisakis* larvae in pigs has recently been described by Japanese and American authors. Ashizawa *et al.* (1973a,b) and Usui *et al.* (1973) found three types of gross lesion in the stomachs of pigs which had become infected with *Anisakis* larva (I) by feeding on fish offal in Japan. Type I lesions were primary mechanical ones, the severity of which was proportional to the numbers of larvae present. Haemorrhage and ulceration of the mucosal and submucosal layers occurred but the muscular and serosal layers were unaffected. In Type II lesions there was an Arthus-type reaction with oedema and cellular infiltration in the mucosal and submucosal layers, connective tissues and muscle layers. Type III lesions were characterized by marked degeneration of the mucous membrane layers, necrosis, ulceration and cellular infiltration. Abscesses were evident in the submucosal layers and slight but diffuse proliferation of connective tissues occurred. Giant cells were seen around dead larvae. The pathology of *Anisakis* larvae in pigs generally resembles that found in other mammals, including man, but differs in the small numbers of granulomata seen and in the presence of allergic oedema in the submucosal layer.

Jackson and Bier and their co-workers have recently investigated the pathology of experimental infections of *Anisakis* larvae in miniature pigs. The majority of larvae penetrated the stomach and were eventually found entirely within the submucosa (Jackson *et al.*, 1976). Ulceration occurred where *Anisakis* penetrated the stomach wall; that there were more ulcers than penetrating larvae suggested to Bier *et al.* (1976a) that larvae could withdraw and repenetrate elsewhere. The area of penetration was haemorrhagic and oedematous and these severe conditions persisted for at least one month. Portions of larvae within the submucosa were surrounded by an acellular cast, probably a nematode secretion. Bier *et al.* (1976b) investigated the blood picture of infected pigs and found a mild eosinophilia one day after infection which receded by day seven. Increased blood levels of amylase, lactic dehydrogenase and bilirubin were also found.

Infection of monkeys, including the Taiwan monkey (*Macaca cyclopis*), was reported by Hsieh and Chen (1970), Wu (1970) and Yamaguchi *et al.* (1970). A brown bear from Alaska was found infected with larval *Anisakis* by Davey (1971b).

In conclusion, mention may be made of the studies of Kitayama *et al.*

(1967) on parasitic granulomata in stray dogs in Hokkaido, Japan. The small granulomatous foci were often caused by foreign bodies or *Toxocara canis* larvae but in some cases unidentified nematode larvae were found. Since all infected dogs were from seaside areas it is possible that *Anisakis* larvae were involved. One case studied in detail revealed *Terranova* (= *Phocanema*) and *Contracaecum* larvae in stomach lesions.

IX. Anisakiasis of Man

A. Geographical distribution and epidemiology

Anisakis has only relatively recently been recognized as an agent of disease in man (see historical account in Jackson, 1975). Human infection with *Anisakis* was classified by Sprent (1969) into one of four categories of anthropozoonosis; for an appraisal of this classification and of the approach of other authors to the concept of "zoonoses" see Williams and Jones (1976).

Hitchcock (1950) found "ascarid" larvae in the stools of 10% of over 100 Alaskan Eskimos examined, one specimen was "with reasonable certainty" identified as *Anisakis*. The first recorded case of a gastrointestinal lesion in man attributable to *Anisakis* occurred in The Netherlands in 1955 (Van Thiel *et al.*, 1960). From 1955 to 1968 a total of 160 confirmed or highly probable cases of anisakiasis were reported in The Netherlands, but since the introduction of freezing regulations for green herring in 1968 (see section XI) only a few new cases have been observed (Van Thiel, 1976). In Japan, anisakiasis was first reported in 1965 (Asami *et al.*, 1965) and subsequently some hundreds of cases have been detected (Oshima in Van Thiel, 1976). Individual cases have also been described from Korea (Kim *et al.* 1971), Denmark (Andreassen, 1970), France (Doby *et al.*, 1975), Belgium (Fain *et al.*, 1969) and the USA (Pinkus *et al.*, 1975).

Anisakiasis might occur in any country where the population at large or ethnic minorities traditionally eat raw or inadequately cooked marine fish or squid harbouring live *Anisakis* larvae. Jackson (1975) reviewed this aspect in a public health context and, with regard to the USA, discussed the possibility of increased risk of infection through increasing consumer interest in "foreign dishes" and the "natural foods movement" with its emphasis on not overcooking food.

Particular eating habits in countries whose people are traditionally at risk may have a bearing on the actual occurrence of anisakiasis. Thus Chen (1971) reported no human cases of anisakiasis in Taiwan and offered the interesting suggestion that the Chinese, unlike the Japanese, eat raw fish at the end of a meal when their stomachs are full and that this may reduce the chances of infection.

Many cases of anisakiasis have been diagnosed from sections of nematodes in tissues resected from the gastrointestinal tract. Given the difficulty of positively identifying anisakine larvae in histological sections it should be stressed that not all of these cases may have been due, in fact, to *Anisakis*; it is possible that one or more of several other anisakine genera were involved.

Where entire larvae recovered from man have been definitely identified as *Anisakis* they appear to fit the description of type (I) larvae (see section IV. E1). In some cases there are indications of a moult to the preadult (fourth) stage.

In Japan, most cases of anisakiasis have occurred in patients aged 20 to 50 years (Oshima, 1972). In both Japan and The Netherlands, recorded rates of infection are higher in men than in women (Ruitenberg, 1970; Oshima, 1972). Tentative suggestions have been made that this sex difference is due to the greater propensity of men to eat raw sea food during the day with alcoholic beverages but no firm conclusion is possible.

In Japan over half the recorded cases of anisakiasis have occurred in the stomach whilst in The Netherlands most cases are intestinal. The reasons for the difference in the localization of larvae are not clear but some suggestions are discussed by Van Thiel (1976). These include differences between the two countries in the quantity of seafood eaten at any one time, the frequency of consumption, stomach fullness, rate of stomach emptying, methods of diagnosis and acidity of gastric juices. Climatic conditions are known to influence gut complaints and, particularly, gastric acidity, so this factor alone may account for the observed differences (Van Thiel, 1976).

B. CLINICAL AND PATHOLOGICAL ASPECTS

As mentioned above, both gastric and intestinal anisakiasis occur in man. The clinical presentation of the two conditions differs in some respects. In gastric anisakiasis sudden stomach pains, nausea and vomiting occur 4–6 hours after the ingestion of raw sea food. Such cases are rarely correctly diagnosed and become chronic with similar manifestations lasting for more than a year. Eosinophils range from 4–41 % in more than half the recorded cases but leucocytosis is absent or slight (Oshima, 1972). Yokogawa and Yoshimura (1967) found occult blood in the gastric juices and stools of 70 % of cases. Anacidity or hypoacidity occurred in many patients; possibly reduced or enhanced gastric secretion enhances the ability of the larvae to penetrate the stomach wall (Asami and Inoshita, 1967).

Intestinal anisakiasis occurs usually within seven days of the ingestion of raw seafood. There is severe pain in the lower abdomen with nausea, vomiting, fever, diarrhoea and occult blood in the stools (Van Thiel *et al*, 1960; Ishikura *et al.*, 1967; Oshima, 1972; Pinkus *et al.*, 1975). Eosinophilia is not usually apparent but in many cases there is a marked leucocytosis. Within the peritoneal cavity 200–500 ml of straw-coloured ascites may be present (Ishikura *et al.*, 1967). The affected region of the small intestine, usually the ileum, is often covered with a fibrous exudate and oedematously thickened causing partial obstruction and proximal distension of the intestine (Van Thiel *et al.*, 1960; Ishikura *et al.*, 1967; Oshima, 1972; Pinkus *et al.*, 1975). Larvae are occasionally found protruding through the gut wall into the abdominal cavity (Ishikura *et al.*, 1967; Van Thiel and Van Houten, 1967). In addition, larvae can be found at other sites in the alimentary tract including the caecum, appendix, colon and rectum, or outside the gut including lymph

nodes, pancreas, greater omentum and mesenteries (Otsuru *et al.*, 1965; Van Thiel and Van Houten, 1967; Yokogawa and Yoshimura, 1967). There have also been some cases of larvae penetrating the wall of the pharynx (Morishita and Nishimura, 1965; Tanaka *et al.*, 1968; Kim *et al.*, 1971).

Japanese workers have classified the lesions found in anisakiasis into five types, the following descriptions of which are based largely on the account of Oshima (1972).

1. A "foreign body response" reaction can occur in the stomach causing benign clinical symptoms with infiltration and proliferation of neutrophils associated with some eosinophils and foreign body giant cells. There may be granulomatous change around the invading larva.

2. In acute intestinal anisakiasis a "phlegmonous reaction (Arthus type)" is frequently seen. There is oedematous thickening of the intestinal wall with massive eosinophilic infiltration of all layers accompanied by other cellular elements. Haemorrhage and fibrin exudation may occur. The larva, which is usually alive or intact, is surrounded by a layer of eosinophils, neutrophils and histiocytes. The larva may be visible in an ulcer in the mucosa but it is often buried within the submucosa, where the reaction is centred, with no ulceration. The mucosa may be oedematous with obliteration of the mucosal folds (Pinkus *et al.*, 1975). The lymphatic channels, including regional lymph nodes, may be involved in the eosinophilic granulomatous response (Pinkus *et al.*, 1975).

3. In chronic gastric and intestinal anisakiasis an "abscess type" lesion may be found. A marked abscess with many eosinophils accompanied by histiocytes and lymphocytes is seen around the degenerating larva and this in turn is surrounded by a granuloma. At the inner layer of the granuloma, necrosis and haemorrhage with eosinophilic infiltration and fibrin exudation or fibrinoid degeneration are seen.

4. In cases of gastric anisakiasis of more than six months duration, an "abscess-granulomatous" type lesion is sometimes found in which a degenerating larva is situated in a reduced abscess surrounded by granulation tissue with slight collagenization. Eosinophilic infiltration of the granuloma is less intensive than in the abscess type lesion and indeed lymphocytes may be dominant instead of eosinophils. The degenerating larva is invaded by eosinophils and surrounded by foreign body giant cells.

5. The most advanced lesion, the "granulomatous" type, occurs when the abscess is completely replaced by granulomatous tissue with eosinophilic infiltration. Only larval debris may be apparent. This type of lesion is seen only occasionally in long standing cases of gastric and intestinal anisakiasis.

C. DIAGNOSIS

The diagnosis of anisakiasis is difficult and in most cases is only correctly made after post-operative pathological examination of the affected part of the gut. Gastric anisakiasis is often diagnosed as stomach ulcer, cancer, tumour or polyp, whilst intestinal anisakiasis is diagnosed mainly as acute appendicitis or as acute regional enteritis (Crohn's disease) (Oshima, 1972). A clinical

diagnosis of gastric anisakiasis can be achieved by fibergastroscopy when the living larva can be observed directly penetrating the stomach wall. Larvae can sometimes be seen by X-ray using the "mucal relief method" (Oshima, 1972). In intestinal anisakiasis the symptoms are alarming and patients are normally operated on within a few days of the onset of disease. Post-operative diagnosis is made by identification of whole larvae or, less reliably, by examining cross sections of larvae in histological sections.

The difficulty of accurate diagnosis of anisakiasis may account for its recent recognition as a disease in man. Before the disease was recognized it seems probable that cases were misdiagnosed, as described above. Thus, according to Oshima (1972), of 1531 cases diagnosed initially as acute regional enteritis, 140 were due to helminths mostly thought to be *Anisakis* whilst 622 were a phlegmonous reaction (Arthus type) with eosinophilia possibly caused by *Anisakis*. The possibility that *Anisakis* was responsible for at least some cases of eosinophilic granulomata in the alimentary tract in the UK and Norway is discussed by Ashby *et al.* (1964) and Jacobsen and Berland (1969). The number of cases of anisakiasis reported from Japan is low in relation to the size of the population at risk and it is suggested by Oshima (1972) that this is due to the difficulty that larvae have in penetrating a healthy human stomach or intestine.

An account of the search for a suitable immunodiagnostic test for anisakiasis in man is given in section X.D.

D. TREATMENT

Because of the difficulty of making a correct diagnosis of anisakiasis and because of the often alarming nature of the symptoms, especially in the acute intestinal condition, treatment is usually by surgery with resection of the affected part. In diagnosed cases of acute gastric anisakiasis, larvae may be removed by means of a gastrofiberscope. In chronic gastric anisakiasis the affected area should be removed surgically in order to avoid possible future allergic exacerbation of the lesion (Oshima, 1972).

If a correct pre-operative diagnosis can be made in intestinal anisakiasis a conservative treatment is recommended with administration of antibiotics and isotonic glucose solution (Oshima, 1972). Prognosis of anisakiasis is good providing post-operative peritonitis is avoided. Van Thiel *et al.* (1960) reported that two patients died due to post-operative enteritis after surgery for intestinal anisakiasis.

X. IMMUNOLOGY AND IMMUNODIAGNOSIS OF ANISAKIASIS

A. GENERAL REMARKS

It has proved difficult to form a coherent view of the extensive literature on this general aspect and it may be helpful to offer some insight into the ways in which this situation may have arisen.

Before a humoral or cell-mediated antibody response can be elicited it may be presumed that at least some worm penetration of the tissues must occur.

Oshima (1972) suggested that *Anisakis* larvae may have difficulty in penetrating the healthy human gastrointestinal tract. However, Asami and Inoshita (1967) experimented with guinea-pigs and regarded the penetration capacity of the larvae as more important than host susceptibility; they noted, as did Lee and Chyu (1970), that some anterior halves of larvae were capable of penetration. Penetration of larvae through the alimentary tract into the body-cavity was recorded in guinea-pigs by Myers (1963) and Asami and Inoshita (1967), and in rats by Nagase (1968b), Young and Lowe (1969), Gibson (1970) and Hashiguchi and Takei (1975). On the other hand, larvae were apparently not recovered from the body-cavity of rabbits by Oyanagi (1967), Ruitenberg (1970, 1971) or Ruitenberg *et al.* (1971). Other examples could be cited but are there real differences between experimental hosts in this regard and can experience with any of them be extrapolated to the situation in man?

Williams and Jones (1976) drew attention to these problems in their interesting review of marine helminths (including *Anisakis*) and human health, and advocated an experimental approach using a far wider range of experimental terrestrial animals of which the physiology or, at least, natural feeding habits are similar to those of man.

In addition to these problems, difficulties of interpretation with regard to immunological and other work on *Anisakis* have arisen due to a combination of one or more of the following factors:

(a) Different workers have used different fish hosts as sources of larval *Anisakis* in different zoogeographical areas.
(b) One or more species of *Anisakis* may have been used, given the difficulties of specific determination of larvae.
(c) Larvae have been subjected to different storage techniques and times, with varying degrees of handling and possible damage.
(d) Some workers have treated their experimental hosts with antiperistaltic drugs, fed them special diets, or starved them before infection.
(e) The mode of infection has varied.
(f) There is often inadequate information on whether or not a moult to the fourth stage has occurred and, if so, in which habitat.

Bier (1976) also noted difficulties of this sort and emphasized that establishment of laboratory life cycles is necessary to provide experimental material in the quantities needed for reproducible results.

With the above reservations in mind, there follows a review of work on the immunopathology and immunodiagnosis of anisakiasis.

B. "HYPERSENSITIVITY" IN RELATION TO PATHOGENESIS

Secondary exposure to antigen of a primed or sensitized animal may result in boosting of the immune response but it may also cause tissue-damaging reactions. These are described as hypersensitivity reactions which may be classified into four types (Coombs and Gell, 1968) to which a fifth type was added by Roitt (1971). The classification is as follows: Type I reaction (anaphylactic, reagin-dependent); Type II reaction (cytotoxic); Type III

reaction (damage by toxic complexes); Type IV reaction (delayed, tuberculin-type, cell-mediated); Type V reaction (stimulation hypersensitivity). Types I, II, III and V are immediate-type reactions which involve humoral antibodies, whilst Type IV involves antibodies bound to the surface of lymphocytes. Specialists in this field do not appear to agree on definitions and mechanisms involved in hypersensitivity reactions. In these circumstances it is difficult to offer a clear picture of the position regarding anisakiasis, especially in view of the fragmentary and equivocal nature of much of the evidence. Type I and Type III reactions at least may be involved.

No special studies have been made of the immune response to infection with *Anisakis* in those animals which feature in the "normal" life cycle, such as crustaceans, squid, fish or marine mammals. Some information on this aspect may be deduced from the histopathological studies that have been made of infected fish and marine mammals (see sections VIII. B and F) but most of the available information comes from investigations on the humoral antibody response of man and other mammals to accidental or experimental infection. Recent work from Japan (Nakamura *et al.*, 1974; Saeki *et al.*, 1973, 1975) suggests that some attention is now being paid to cell-mediated immunity; these brief reports are in the Japanese language and in the form of "abstracts of meetings" so no further comment is possible here.

Rodenburg and Wielinga (1960) suggested the possible "allergic" nature of anisakiasis of man and since that time a number of Dutch and Japanese workers have discussed this aspect from observations not only in man but also in other mammals including experimentally infected pigs, guinea-pigs, cats, dogs and rabbits.

In an attempt to explain the apparent rareness of anisakiasis of man, several workers have investigated the "double-hit theory" apparently first postulated by Roskam (see Ruitenberg, 1970). The view is that within a certain period a second *Anisakis* larva needs to penetrate the gastrointestinal tract at approximately the same site where a first larva has already penetrated in order to produce severe reaction. In a series of papers Kuipers and co-workers (Kuipers, 1962, 1964; Kuipers *et al.*, 1963) investigated this suggestion of a local hypersensitivity resulting from primary infection using experimentally infected rabbits. From these studies and observations on the proximity of lesions in resected tissue samples from man, Kuipers (1964) concluded that the "double-hit theory" was valid. Ruitenberg and co-workers (Ruitenberg, 1970, 1971; Ruitenberg *et al.*, 1971) reviewed the evidence and conducted further studies on experimentally infected rabbits. Ruitenberg (1971) concluded that a local hypersensitivity may be produced by a previously penetrated larva and that another larva penetrating at this site may provoke severe reaction with necrosis, oedema and haemorrhage. Significantly, however, he found that the same reaction could be induced by one larva in a previously uninfected rabbit; he suggested, therefore, that human anisakiasis may be caused by a single larva.

As an alternative to the controversial "double-hit theory", Oshima (1972) discussed the "exacerbation theory" of Kozima *et al.* (1966) to explain severe reaction in man. Exudate from a dead larva may react with the newly formed

granuloma which has been previously sensitized by the metabolic products of the living larva. Similarly, from experiments with rabbits, Oyanagi (1967) suggested that the causative agents of tissue reaction may be the excretions and secretions (ES) of living larvae or the decomposition products of dead larvae. Oshima (1972) pointed out that the "exacerbation theory" does not explain the "phlegmonous type (Arthus type) reaction" seen in acute intestinal anisakiasis of man and discussed this problem further. Discussion of a supposed local hypersensitivity in the gastrointestinal tissues of man appears to founder on uncertainty as to how long the hypersensitivity takes to develop and, importantly, for how long it persists.

Pinkus *et al.* (1975) noted that the marked oedema, eosinophilia and granuloma formation in anisakiasis appear out of all proportion to the small size of the worm; they suggested that spreading lymphangitis may be involved in pathogenesis. In a human case studied by Pinkus and co-workers, the eosinophilic and granulomatous response involved lymphatic channels including the regional lymph nodes. Antigens released by the degenerating larva may be carried through the lymphatics of tissue which has already been sensitized by material from the live parasite. As these substances progress along the course of lymphatics, they may elicit an eosinophilic granulomatous thrombolymphangitis. Obstruction of lymphatic channels by this mechanism may contribute to the marked oedema.

From intradermal tests using somatic or ES antigen of larval *Anisakis*, Kobayashi *et al.* (1968b) reported higher skin sensitivity in persons predisposed to allergic diseases (e.g. asthma, urticaria, eczema and dermatitis) than in "normal" persons. Kobayashi *et al.* (1968c) reported that the ES antigen showed a higher specificity than the somatic antigen in skin tests. Suzuki *et al.* (1970, 1971) used a haemoglobin extract of larval *Anisakis* as antigen in intradermal tests on man; tests on over 1000 Japanese showed positive reactions to be more common among people living by the sea than in those inhabiting mountainous regions. From these results and special observations on clinical symptoms and pathology in 35 cases, Suzuki *et al.* suggest that anisakiasis may be divided into two forms, mild and fulminant: the mild form shows negative intradermal reaction and may be the result of primary infection; the fulminant form shows positive intradermal reaction and may be an allergic reaction induced by secondary infection. However, conclusions arising from the use of skin tests should be viewed with caution; false positives may arise because antigenic material itself may be capable of degranulating mast cells and releasing histamine, without there being any need for it to react with the specific antibodies found only in infected individuals (Terry, 1968).

C. ANTIBODY RESPONSE

Ascaridoid infections in general are characterized by a good response of all major classes of immunoglobulins. A distinctive feature is the unusually high levels of IgE and IgG antibodies that are induced in man and animals. High

levels of specific IgE are produced not only by active ascaridoid infection but also by sensitization with either crude extracts or semi-purified preparations from the nematodes. The ascaridoids also show a marked degree of antigenic cross-reactivity between different species and genera (Soulsby, 1976).

These general statements may apply to accidental infection with *Anisakis* in man and some support for this view has come from animal experiments. Taniguchi (1970a) injected phosphate-buffered saline extracts of larval or adult *Anisakis* with Freund's complete adjuvant into the foot pads of rabbits. After six days homocytotropic antibody was found in three of five rabbits and persisted for 14 to 20 days. In rabbits infected with three oral doses of 60 larvae at two-day intervals, homocytotropic antibody was detected after ten days in two of three rabbits and persisted for about ten days. The maximum titre for homologous passive cutaneous anaphylaxis (PCA) was reached after a latent period of 72 hours and was present at 144 hours. Homocytotropic activity was heat labile and inactivated by reduction and alkylation. From their work on mice experimentally infected with larval *Anisakis*, Kobayashi *et al.* (1972, 1974) concluded that the immunoglobulin responsible for the observed mast cell degranulation was mostly reaginic in nature. Reagins are generally recognized as belonging to the IgE class (Eisen, 1974).

Sato *et al.* (1975) reported on possible aetiological mechanisms that operate in anisakiasis. Their work is interesting with regard to the mast cell degranulation studies of Kobayashi *et al.* (1972, 1974) and to immediate-type hypersensitivity (anaphylactic) reactions in relation to IgE described by Eisen (1974). Sato *et al.* (1975) sensitized guinea-pigs with larval *Anisakis* and examined, *in vitro*, the anaphylactic response of segments of ileum. Segments contracted vigorously when treated with whole worm extract of larval *Anisakis* but there was no response when the treatment was repeated. A lower response was obtained with larval extracts of *Terranova* (= *Phocanema*) and *Contracaecum* (? *Thynnascaris*).

The eosinophil leucocyte with granuloma formation is a regular feature of many helminth infections including *Anisakis* in man and animals. Poynter (1966) discussed evidence which suggests that the eosinophil is attracted by, and concerned with, histamine liberated as a result of tissue damage. More recently, Warren (1974, in discussion on schistosomiasis) briefly discussed the "eosinophil chemotactic factor of anaphylaxis" in relation to granuloma formation and IgE but the picture is not yet clear.

The formation of precipitate in the head region of larval *Anisakis* incubated in human sera may not be an immune phenomenon since it was observed in both "immune" and "control" sera (Van Thiel, 1967). The precipitate may involve non-specific esterase, high concentrations of which occur in the "excretory gland" but which does not appear to be antigenic (Ruitenberg, 1970; Ruitenberg and Loendersloot, 1971a, b).

D. IMMUNODIAGNOSIS

Search for a sensitive and reliable immunodiagnostic test for anisakiasis of man has been hampered largely by the common problem of cross-reactivity

with other genera and species in the superfamily Ascaridoidea and even with other, less closely related, helminths. Cross-reactivity has been a feature of a variety of tests (intradermal, complement fixation, immunofluorescent, haemagglutination) using various antigens; most of the published information has been adequately reviewed by Ruitenberg (1970), Oshima (1972) and Williams and Jones (1976).

Antigenic analyses, conducted largely by Japanese workers, also reveal evidence of cross-reactivity. For example, from tests using heterologous and homologous antisera, Taniguchi (1966) reported cross-reactivity between larval *Anisakis*, adult *Toxocara* and adult *Ascaris*. Starch gel electrophoretic analysis of larval *Anisakis* and adult *Ascaris* suggested that only one component of more than 20 from each of the nematodes was species specific (Suzuki, 1968).

Taniguchi (1970b) sensitized rabbits with saline extracts of either larval *Anisakis* (from mackerel) or adult *Anisakis* (from dolphin); in serological tests, higher precipitation titres occurred with the adult extract. Electrophoresis demonstrated more than six antigenic components in the larval extract and more than five in the adult extract; Ouchterlony tests failed to distinguish between adult and larval extracts. Tsuji (1975) used immunoelectrophoresis to study homologous and heterologous antibody-antigen reactions of the antigens from various helminths; he stated that specific bands were identified for larval *Anisakis* (from mackerel and dolphin) and that larval and adult antigens of *Anisakis* are different.

Perhaps the most promising immunodiagnostic test to have been reported is that of Suzuki *et al.* (1974) using an indirect fluorescent antibody test with haemoglobin extracted from larval *Anisakis* as antigen. The haemoglobin antigen is claimed to be stable and sensitive; the extraction technique and antigenic characteristics were reported in a series of earlier papers (Suzuki *et al.*, 1969, 1970, 1971; Sato *et al.*, 1973, 1974; Shiraki *et al.*, 1973). The method used is essentially that of the soluble antigen fluorescent antibody test of Toussaint and Anderson (1965), with coupling of the protein to fine agarose beads as suggested by Porath *et al.* (1967). The haemoglobin is extracted from perienteric fluid of larval *Anisakis* by ammonium sulphate precipitation and DEAE cellulose column chromatography. Coupling of the water-soluble haemoglobin to cyanogen bromide-activated Sepharose 4B eliminated difficulties associated with removing non-reacting test serum, and hence conjugate, from the cellulose acetate matrix. The antigen-coupled gel retained its antigenicity for over two months at 4°C; greater stability was obtained by lyophilizing the material in a 2% saccharose and 0·5% BSA solution. In cross-reactivity experiments with *Ascaris* and *Toxocara* infections in rabbits, the *Anisakis* haemoglobin antigen was more specific than whole worm antigen. The success of this or any other immunodiagnostic technique for general use will depend on the free availability of antigen in sufficient quantities and the careful standardization of reagents.

XI. Prevention of Accidental Infection with *Anisakis*

The importance of *Anisakis* as a human pathogen has resulted in much research into methods of locating larvae in fish, particularly in the flesh, and killing them. Larvae are colourless and normally tightly coiled in a spiral some 3 mm in diameter which makes them extremely difficult to find within the flesh. In the fish processing industry, candling of fish fillets has been widely used to detect larval nematodes. Candling involves examining fillets over a ground glass screen illuminated from below by a fluorescent light. Although candling is a reasonably efficient method of locating the larger and more highly coloured larvae of *Phocanema* it is, in our experience, of only limited value in the detection of *Anisakis* larvae. In fish with dark-coloured flesh it is virtually useless. An improvement in the candling technique was suggested by Power (1961) who sliced fillets longitudinally before candling; such a method can be of only limited commercial value.

Most larval *Anisakis* within the flesh of fish are located within the hypaxial muscles which surround the body cavity (see, for example, Templeman *et al.*, 1957; Novotny and Uzmann, 1960; Smith and Wootten, 1975). The practice of block filleting in which the epaxial muscles alone are removed from the fish will result in only a relatively small proportion of the *Anisakis* larvae in a fish remaining in the final product.

A number of other methods of locating nematode larvae within the flesh of fish has been developed but these are of value only in scientific investigations or in small-scale samplings of commercial catches. The simplest method is dissection of the flesh but the most efficient and reliable technique is digestion of the flesh using a mixture of pepsin and hydrochloric acid (Stern *et al.*, 1958; Smith and Wootten, 1975). Larval *Anisakis* are extremely resistant to digestion even at temperatures up to 50°C presumably because of the tough nature of the cuticle. The superiority of a digest method over dissection was demonstrated by Stern *et al.* (1958) who found that digest recovered 21·9% more *Anisakis* larvae from the flesh of chum salmon (*Oncorhynchus keta*) than manual dissection. Similarly, low numbers of larval *Anisakis* in the flesh of herring reported by Khalil (1969) and Davey (1972a) compared with those given by Smith and Wootten (1975) are certainly due to the use of a digest technique by us.

Oishi *et al.* (1971) used several techniques to determine the number of larval *Anisakis* in the flesh and viscera of a number of fish and squid species. In contrast to other workers they did not find enzymatic digestions suitable; bacterial decomposition was also unsuitable. Larvae were easily detected by the compression of musculature between two glass plates but this method was less suitable for dark muscle or viscera. Autolysis was a better method for recovering larvae from the viscera.

Because it is impossible to detect all larval *Anisakis* within the flesh of fish without rendering it useless for human consumption, methods must be devised of killing larvae before it is eaten. In countries such as the United Kingdom, where fish products are usually well cooked before consumption

anisakiasis is apparently rare. Larval *Anisakis* are killed very quickly at temperatures above 60°C both in water (Kawada, 1968) and in fish flesh (Davey, 1972b). At temperatures below this the death of larvae cannot be guaranteed.

In some smoking processes, for example the kippering of herring, the temperature of the fish flesh does not rise above 40°C so live larvae may still be present. Khalil (1969) found that cured and smoked split herring contained live larvae, although deboned and block-filleted herring in which the hypaxial muscles are removed contained no larvae. In a review of Dutch smoking procedures, Ruitenberg (1970) suggested that kippered and golden herring which are heated to about 28°C and 40°C respectively during the smoking process would contain live larvae whereas red herring heated to 60°C would be safe. Hot smoking processes employed in the United Kingdom for the preparation of salmon, white fish and some herring products involve temperatures of up to 80°C which would result in the death of all larvae.

Freezing of infected fish probably represents the only effective way of controlling anisakiasis where fish are eaten raw or lightly salted. Gustafson (1953) showed that at −17°C all larval *Anisakis* were killed within 24 hours. Commercial freezing of 100 lb blocks of herring at about −30°C for 16 hours followed by storage at −12°C killed nearly all larvae within 24 hours and any survivors apparently within one week. In The Netherlands, freezing regulations were introduced to prevent anisakiasis through consumption of lightly cured herring. Under these regulations herring must be frozen to −20°C within 12 hours and kept at not higher than this temperature for at least 24 hours (Ruitenberg, 1970). Ruitenberg was unable to infect rabbits with larval *Anisakis* treated in this way. Frozen fish may be safely used for smoking or other processes which in themselves will not kill all larvae. In some fish, notably herring, there is a migration of *Anisakis* larvae from the viscera into the flesh if fish are not gutted immediately after death (Van Thiel *et al.*, 1960; Vik, 1966; Smith and Wootten, 1975). Such migrations could be prevented by shipboard freezing soon after capture. Trash marine fish to be used as feed for cultured fish should also be frozen before use in order to prevent transmission of larval *Anisakis* (Bassleer *et al.*, 1973; Wootten and Smith, 1975).

Larval *Anisakis* are very resistant to curing processes. Van Thiel *et al.* (1960) showed that larvae could survive in a saturated salt solution for 1¾ hours whilst Khalil (1969) reported that they survived in such a solution for less than one day. At a concentration of 50 g litre^{-1}, however, larvae survived for a "very long time". In The Netherlands, salted herring may be divided into "heavily salted" (kept in a brine solution of a strength of at least 20° Baumé) and "lightly salted" (kept in a solution of less than 20°Baumé). In the former, larvae are killed within ten days but in the latter larvae can survive for long periods thus necessitating the use of freezing regulations (Ruitenberg, 1970). Grabda (1973) reported that larval *Anisakis* can survive for 7–35 days in a brine solution of 15°–19° Baumé. Dry salt will kill larvae in less than ten minutes (Khalil, 1969) but a suitable concentration must be reached throughout the body of the treated fish. As an example of this, Khalil (1969) noted

that in lightly cured herring kept in coarse dry salt for six days only 2% of the larvae from females survived but 82% from males were still alive. Khalil attributed this difference to the greater moisture content of soft roes lessening the effect of the salt.

Larval *Anisakis* are also able to withstand the marinating process for herring as used in Europe. Marination involves subjecting herring to an acetic acid and brine mixture in order to denature the proteins. The duration of the process may vary as does the ratio of herring to solution in the baths. This ratio may vary from 1 part herring to 2 parts solution to 2 parts herring to 1 part solution. Ruitenberg (1970) found that a herring/solution ratio of 1:1 and an initial concentration of 4% acetic acid and 6% sodium chloride would not kill *Anisakis* larvae after 26 days, although earlier Dutch work had shown that marination for 70 days in this solution was effective. The earlier work also showed that with a herring/solution ratio of 2·2:1 and an initial concentration of 7% acid and 15% salt, the ability of larvae to penetrate agar gel *in vitro* was reduced from 100% after six days to only 3% at 30 days. Priebe *et al.* (1973) concluded that at a salt concentration of 5·8% and pH not greater than 4·1, herring should remain in solution for at least 30 days. At 5·0% salt concentration the period should be 35 days. When these conditions cannot be fulfilled, only deep-frozen herring should be used.

Dutch and Japanese workers have experimented with the use of irradiation to render herring safe. Van Mameren and Houwing (1967) found that in aqueous solution all *Anisakis* larvae were killed in a salt concentration of 6% and a radiation dose of 1·0 Mrad (at 0·006 Mrad min $^{-1}$). Many larvae survived at lower salt concentrations and radiation doses. Larvae in 5% salted vacuum packed herring irradiated at 0·3 and 0·6 Mrad at 0·003 Mrad min $^{-1}$ survived in large numbers. Higher dosage rates unacceptably altered the flavour of the herring products. Oishi *et al.* (1972b) concluded that the use of irradiation in fish processing is not practicable because decomposition of fish products occurs at doses over 0·6 Mrad.

Anisakis larvae are remarkably resistant to a wide variety of chemicals, spices and food additives (Oshima, 1972; Oishi *et al.*, 1974) many of which are used in the preparation of raw fish dishes in Japan. Kato (in Oshima, 1972) found that larvae were killed in two hours by a 5% solution of commercial wasabi powder, a Japanese fish seasoning, but suggested that larvae in fish would not be killed during the usual conditions of use of wasabi.

In many countries, particularly in the Far East, the practice of eating raw or very lightly salted fish is widespread and deeply rooted in the way of life of the population. For these reasons, public health education programmes designed to draw attention to the dangers of anisakiasis will be unlikely to achieve great success. In such countries it would be extremely difficult to apply preventive legislation such as compulsory freezing of fish products because the fishing industry is often made up of widely scattered small units of production and the variety of fish used for human consumption is very great. It is probable that in some of these countries the elimination of anisakiasis cannot be separated from their economic and social development which may eventually enable effective preventive measures to be undertaken.

XII. *ANISAKIS* AS A BIOLOGICAL TAG FOR FISH

As the need for a more rational use of the dwindling fish resources of the world increases the accurate distinction of fish stocks becomes of paramount importance. Fisheries scientists have relied mainly on mechanical tagging procedures to determine the movements of fish but such methods are time consuming and expensive with a very poor return rate of tagged fish. In recent years increasing use has been made of parasites as biological tags for fish.

The use of parasites in this manner depends on the fact that different stocks of a fish species may have significantly differing rates of infection of one or more parasite species. Thus, parasitological examination of a sample of fish may reveal from which stock it is derived. If a quantitative assessment of the infection rate of different stocks can be made, it is at least theoretically possible to identify the individual components of mixed stocks.

A number of conditions which preferably must be fulfilled before a parasite can be successfully used as a biological tag were listed by Kabata (1963). In summary these are as follows:

1. A parasite should be common in one stock but rare or absent in other stocks of fish.
2. The parasite should include only the host species under study in its life cycle.
3. Infections of the host fish with the parasite must be of reasonably long duration.
4. The infection of the parasite must remain relatively stable within and between years.
5. Environmental conditions throughout the area studied must be within the physiological range of the parasite.

The use of larval *Anisakis* as a biological tag for fish may be criticized in relation to the second of these conditions. Kabata (1963) acknowledged that the usefulness of a parasite with two or more hosts in its life cycle will depend on the distribution and abundance of all the host populations. *Anisakis* has crustacean, squid, fish and mammalian hosts, and within any given area there may be a number of species of each group acting as hosts. It is doubtful that there is any geographical area where the circulation of *Anisakis* amongst its different hosts is adequately defined, so the value of the parasite as a tag is reduced.

Few workers who have used larval *Anisakis* as biological tags have attempted a specific identification of the larvae. The possibility always exists that at least two species of *Anisakis* occur in the host under investigation and this greatly complicates the use of the parasite as a tag. The incidence of infection of larval *Anisakis* in fish probably varies greatly over a relative short period (Wootten and Waddell, 1977) and this also might affect the usefulness of the parasite as a tag in the long term.

Large differences in the abundance of larval *Anisakis* in a fish species from different areas are well known and not surprisingly, therefore, larvae have

been used as biological tags, either alone or in conjunction with other parasites, by a number of workers.

Bishop and Margolis (1955) found that the intensity of infection of Pacific herring (*Clupea pallasi*) with larval *Anisakis* differed significantly between geographical areas off British Columbia. Within the Gulf of Maine, Sindermann (1961a) found that Atlantic herring (*Clupea harengus*) spawning on Georges Bank and off Nova Scotia were distinguishable by differences in the prevalence of larval anisakines and cestodes. In juvenile herring from the coast of Maine, Sindermann found that one-year-old fish from eastern Maine were more heavily infected with larval anisakines than fish from western Maine. This difference persisted in two-year-old herring although "western" fish showed a proportionally greater increase in infection which indicated a possible westward movement of herring that had spent their first year off the east coast of Maine. It is possible that the herring examined by Sindermann were parasitized by both larval *Anisakis* and *Thynnascaris*.

Considerable differences in the incidence of infection with larval *Anisakis* of herring from the Gulf of St. Lawrence and around Newfoundland were noted by Parsons and Hodder (1971). Herring fished near the Magdalen Islands in spring and autumn and near Gaspé in summer had a very similar level of infection to those fished off south-western Newfoundland in winter, which suggested that all these fisheries were on a common stock of herring. A similar incidence of infection with larval *Anisakis* in herring from north-eastern Nova Scotia and the Banquereau-Sable Island areas suggested an inshore–offshore migration of fish between these areas.

From a study of the level of infection with *Anisakis simplex* larvae in addition to differences in otolith type and gonad maturity, Grabda (1974) was able to distinguish between stocks of herring in the southern Baltic. Only those spring-spawning herring which spawned in this area from November to May were heavily infected with larvae. These fish acquired their infections in the North Sea during the summer months. Herring which had spent all their lives within the Baltic were only occasionally infected.

The use of *Anisakis* larvae as a biological tag may be of value in the separation of adult herring stocks around the British Isles. The level of infection of herring from these waters with larval *Anisakis* varies greatly between different localities (Khalil, 1969; Davey, 1972a). In general, the abundance of larval *Anisakis* decreases from north to south in these waters.

In the northern North Sea, MacKenzie and Wootten (1973) showed that the level of infection of whiting (*Merlangius merlangus*) with larval *Anisakis* (probably *A. simplex*) and the plerocercoids of the trypanorhynch cestode *Gilquinia squali* was highest in central offshore areas and lowest off the Scottish East coast, with an intermediate level of infection around the Shetland Isles. These observations are not inconsistent with the results of mechanical tagging which indicate distinct inshore and offshore whiting populations in the northern North Sea with an area of mixing around the Shetlands (Hislop and MacKenzie, 1976).

The use of parasites, including larval anisakines, as biological tags for redfish (*Sebastes marinus*) was suggested by Sindermann (1961b), who found

larvae to be much less abundant in fish from offshore areas along the coasts of Maine and Nova Scotia than in those from inshore areas. A comparison of the parasite fauna of redfish from different fishing grounds in the general area suggested little mixing of stocks.

Anisakis larvae have also been used as biological tags for fish populations in limited inshore areas. Gibson (1972) used a number of parasites of flounders (*Platichthys flesus*), including *Anisakis* sp. larvae, as biological tags to distinguish between populations in a small area off the Scottish East coast. Flounders from the estuaries of the Rivers Dee and Ythan could be distinguished from each other and from fish caught in the purely marine habitat of Aberdeen Bay. *Anisakis* sp. larvae were much more abundant in the flounders caught in the open sea, probably because more infected crustacean hosts were available in this area. Kilambi and De Lacy (1967) were able to distinguish between spawning populations of surf smelt (*Hypomesus pretiosus*) on the coast of Washington State and in Puget Sound by the levels of infection with larval *Anisakis*. Fish from the outer coast were more heavily infected than those in Puget Sound, from which it was deduced that they had fed in the open sea where the cetacean hosts were abundant.

The distinction between Atlantic salmon (*Salmo salar*) of European and North American origin in the high seas fishery at Greenland has assumed great importance in recent years as a result of the demand for conservation of this species. Nyman and Pippy (1972) found that *Anisakis simplex* larvae, in conjunction with the abundance of the cestode *Eubothrium crassum* and biochemical features of the host fish, could be used as a biological tag. In biochemically identified salmon of North American origin caught at Greenland there appeared to be significantly fewer *Anisakis* larvae than in European fish. The numbers of larvae in mechanically tagged and biochemically identified N. American salmon were similar. In an earlier publication, Pippy (1969) used parasites, including larval *Anisakis*, to distinguish between salmon populations caught off the Canadian East coast. It appeared to be possible also to distinguish between salmon which had migrated to Greenland and those which had not. Konovalov (1967) used a number of parasites including larval *Anisakis* sp. to identify local stocks of Pacific salmon (*Oncorhynchus*) in the eastern USSR.

Sindermann (1961a) suggested that parasites will seldom provide definite answers to problems of the identification of fish populations and migrations but that in conjunction with other techniques, such as mechanical tagging and meristic studies (to which must now be added serological studies), they may provide a significant contribution. Within these limitations *Anisakis* larvae may be useful as a biological tag.

XIII. Conclusions

This review of the literature on *Anisakis* and anisakiasis (*sensu stricto*) has revealed many gaps in our knowledge to which attention has been drawn in various sections. The aim of the following paragraphs is to highlight only certain of the aspects which appear to warrant further investigation.

Reference to *Anisakis* in the more recent literature as "herring-worm" is misleading and should be avoided in future; in at least the European North Atlantic, recent work suggests that whilst larval *Anisakis* is common in herring it is equally, if not more, abundant in other fish species, including blue whiting (*Micromesistius poutassou*) (Wootten and Smith, 1976, and work in progress).

Despite Davey's (1971a) revision of the genus *Anisakis* there are still taxonomic problems, including the distinction between *A. simplex* and *A. typica*. The generic diagnosis of *Anisakis* should be re-examined in the light of Petter's (1972) redescription of *A. insignis*.

Anisakis larva (I) from North Atlantic waters has been cultured *in vitro* and shown to develop into *A. simplex*. There is a need to culture the other "larval types" in order to confirm that they do, in fact, represent *Anisakis* and, if so, to determine which species they represent.

Whilst euphausiids appear to be important crustacean hosts of *Anisakis* at least in offshore areas, further work may show other crustaceans to be significant, possibly in inshore waters. Further investigation may show squid to be of greater significance in the transmission of *Anisakis* to final hosts than the literature indicates, bearing in mind the importance of squid in the diet of many cetacean species (Clarke, 1966). Several aspects of development remain obscure. For example, the course of events in crustacean hosts has not been fully established, including whether or not the second moult occurs in these hosts.

There is a dearth of information on the behaviour, physiology and biochemistry of different stages in the life history of *Anisakis*. Such information as exists is concerned mainly with the larval stage from fish. Rather more physiological work has been done on the related genus *Phocanema* and similar experimental approaches to *Anisakis* may prove to be rewarding.

For example, detailed studies by K. G. Davey and co-workers on the moulting process in *Phocanema* have provided evidence that an "ecdysial hormone" released from neurosecretory cells of the ganglia associated with the nerve ring may act on the "excretory gland", causing activation and release of "leucine aminopeptidase" and other enzymes (Davey, 1976, and references therein).

Certain volatile ketones and alcohols are produced by larval *Phocanema* and the intriguing suggestion has been made that these may function as local anaesthetics as the worm burrows through fish muscle (Ackman and Gjelstad, 1975; Ackman, 1976).

In view of the taxonomic importance of the "excretory system" in ascaridoid nematodes (Hartwich, 1974) it is unfortunate that its physiological functions are so poorly understood; *Anisakis* may prove to be a useful experimental model for investigation of these functions.

With regard to accidental infection of man with *Anisakis*, further study might be made of a possible allergic predisposition to severe gastrointestinal reaction.

In addition to *Anisakis*, certain other marine anisakine genera (e.g. *Phocanema, Contracaecum*) have been implicated as causative agents of human

anisakiasis (*sensu lato*). In these circumstances there is a need for reliable methods of differential diagnosis.

There is much scope for future investigation.

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References

Many of the Japanese papers cited in this reference list are provided with an English summary. Complete translations of some of the Japanese papers were obtained from Translation Services, Canada Institute for Scientific and Technical Information, National Research Council of Canada.

Ackman, R. G. (1976). Volatile ketones and alcohols of codworms (*Terranova decipiens*) from axenic culture and fresh fish muscle. *Journal of the Fisheries Research Board of Canada* **33**, 2819–2821.

Ackman, R. G. and Gjelstad, R. T. (1975). Gas-chromatographic resolution of isomeric pentanols and pentanones in the identification of volatile alcohols and ketones in the codworm *Terranova decipiens* (Krabbe, 1878). *Analytical Biochemistry* **67**, 684–687.

Akhmerov, A. K. (1951). (Some data on the parasites of Alaska pollock.) *Izvestiya Tikhookeanskogo nauchno-issledovatel'skogo instituta rȳbnogo khozyaistva i okeanografii* **34**, 99–104. (In Russian.)

Alekseev, V. M. and Smetanina, Z. B. (1968). (Nematodes of fish-eating birds of the Rimsko-Korsakov Islands.) *In* (Helminths of animals of the Pacific Ocean) (K. I. Skryabin and Y. L. Mamaev, eds), pp. 97–104. Izdatel'stvo "Nauka", Moscow. (In Russian.)

Aligadzhiev, A. D. and Altaev, A. Kh. (1973). (Nematodes of fish of the inland waters of Dagestan, USSR.) *Materialy Nauchnykh Konferentsii Vsesoyuznogo Obshchestva Gel'mintologov* **1971–1972**, No. 25, 6–12. (In Russian.)

Anderson, R. C., Chabaud, A. G. and Willmott, S. (1974). General introduction. Glossary of terms. Keys to subclasses, orders and superfamilies. "CIH Keys to the Nematode Parasites of Vertebrates", No. 1, 17 pp. Commonwealth Agricultural Bureaux, Farnham Royal, Bucks, England.

Andreassen, J. (1970). The first known case of anisakinosis in Denmark. *Norwegian Journal of Zoology* **18**, 105.

Arai, H. P. (1969). Preliminary report on the parasites of certain marine fishes of British Columbia. *Journal of the Fisheries Research Board of Canada* **26**, 2319–2337.

Areán, V. M. (1971). Anisakiasis. *In* "Pathology of protozoal and helminthic diseases with clinical correlation" (R. A. Marcial-Rojos, ed.), pp. 846–851. Williams and Wilkins, Baltimore, USA.

Asami, K. and Inoshita, Y. (1967). Experimental anisakiasis in guinea pigs:

factors influencing infection of larvae in the host. *Japanese Journal of Parasitology* **16**, 415–422.

Asami, K., Watanuki, T., Sakai, H., Imano, H. and Okamoto, R. (1965). Two cases of stomach granuloma caused by *Anisakis*-like larval nematodes in Japan. *American Journal of Tropical Medicine and Hygiene* **14**, 119–123.

Ashby, B. S., Appleton, P. J. and Dawson, I. (1964). Eosinophilic granuloma of gastro-intestinal tract caused by herring parasite *Eustoma rotundatum*. *British Medical Journal* **1** (5391), 1141–1145.

Ashizawa, H., Nosaka, D., Tateyama, S. and Usui, M. (1973a). (Studies on swine gastric anisakiasis. II. Macroscropic lesions.) *Bulletin of the Faculty of Agriculture, Miyazaki University* **20**, 179–189. (In Japanese.)

Ashizawa, H., Nosaka, D., Tateyama, S. and Usui, M. (1973b). (Studies on swine gastric anisakiasis. III. Histopathological findings.) *Bulletin of the Faculty of Agriculture, Miyazaki University* **20**, 191–201. (In Japanese.)

Bakke, T. A. and Baruš, V. (1975). Studies of the helminth fauna of Norway. XXXVI. The common gull, *Larus canus* L., as final host for Nematoda. 1. Qualitative and quantitative data on species of Ascaridoidea (Railliet and Henry, 1915). *Norwegian Journal of Zoology* **23**, 183–191.

Bassleer, J., Puylaert, F. and Beneden, P. van (1973). Une menace d'anisakose. Présence de larves de l'ascaride des marsouins et des phoques dans une salmoniculture ardennaise. *Revue médicale de Liège* **28**, 273–276

Baylis, H. A. (1944). "*Capsularia marina*" and the Ascaridae of marine hosts. *Parasitology* **36**, 119–121.

Belogurov, O. I., Leonov, V. A. and Zueva, L. S. (1968). (Helminth fauna of fish-eating birds (gulls and guillemots) from the coast of the Okhotsk Sea.) *In* (Helminths of animals of the Pacific Ocean.) (K. I. Skryabin and Y. L. Mamaev, eds), pp. 105–124. Izdatel'stvo "Nauka", Moscow. (In Russian.)

Berland, B. (1961). Nematodes from some Norwegian marine fishes. *Sarsia* **2**, 1–50.

Beverley-Burton, M., Nyman, O. L. and Pippy, J. H. C. (1977). The morphology, and some observations on the population genetics of *Anisakis simplex* larvae (Nematoda: Ascaridata) from fishes of the North Atlantic. *Journal of the Fisheries Research Board of Canada* **34**, 105–112.

Bier, J. W. (1976). Experimental anisakiasis: cultivation and temperature tolerance determinations. *Journal of Milk and Food Technology* **39**, 132–137.

Bier, J. W., Jackson, G. J., Earl, F. L. and Knollenberg, W. G. (1976a). Experimental anisakiasis in pigs: gross and microscopic pathology with larval *Anisakis* sp. and *Phocanema* sp. nematodes from fishes. (Abstract.) *Transactions of the American Microscopical Society* **95**, 264–265.

Bier, J. W., Jackson, G. J. and Gerding, T. A. (1976b). Experimental anisakiasis in pigs: hematology and clinical chemistry with larval *Anisakis* sp. and *Phocanema* sp. nematodes from fishes. (Abstract.) *Transactions of the American Microscopical Society* **95**, 265.

Bishop, Y. M. M. and Margolis, L. (1955). A statistical examination of *Anisakis* larvae (Nematoda) in herring (*Clupea pallasi*) of the British Columbia coast. *Journal of the Fisheries Research Board of Canada* **12**, 571–592.

Bisseru, B. (1975). Strongyloidiasis and anisakiasis. *In* "Diseases Transmitted from Animals to Man" (W. T. Hubbert and W. F. McCulloch, eds), pp. 601–610. Charles C. Thomas, Springfield, Illinois, USA.

Blessing, M. H. and Peitz, G. (1970). Anisakisbefall in Gefangenschaft gehaltener mariner Säuger. *In* "Verhandlungsbericht des XII Internationalen Symposiums über die Erkrangungen der Zootiere, 6–10 Mai, 1970, in Budapest" (D. Matthias

ed.), pp. 283–286. Akademie-Verlag, Deutsche Akademie der Wissenschaften, Berlin.

Brand, T. von (1966). "Biochemistry of Parasites" Academic Press, New York and London.

Brian, L. (1958). Ricerche sul ciclo biologico e l'ecologia di *Ascaris capsularia* Rud. *Atti dell'Accademia ligure di scienze e lettere* **14**, 249–263.

Bryant, C. (1975). Carbon dioxide utilization, and the regulation of respiratory metabolic pathways in parasitic helminths. *Advances in Parasitology* **13**, 35–69.

Chen, E. R. (1971). Species of marine fishes found with *Anisakis* larvae in Taiwan. (Abstract.) *In* "Proceedings of the 7th Southeast Asian Seminar on Tropical Medicine and Public Health: Infectious Diseases of the Gastrointestinal System in Southeast Asia and the Far East, Taipei, Taiwan, 28 September–2 October, 1970" (J. H. Cross, ed.), p. 301. SEAMO Central Coordinating Board for Tropical Medicine and Public Health Project (1971), Bangkok, Thailand.

Cheng, T. C. (1976). The natural history of anisakiasis in animals. *Journal of Milk and Food Technology* **39**, 32–46.

Chitwood, B. G. (1974). Life history. General discussion. *In* "Introduction to Nematology" (B. G. Chitwood and M. B. Chitwood, eds), pp. 243–245. University Park Press, Baltimore, London, Tokyo.

Clarke, M. R. (1966). A review of the systematics and ecology of oceanic squids. *Advances in Marine Biology* **4**, 91–300.

Clayton, D. (1975). Farming green turtles. *Fish Farming International* **2**, 9–11.

Collard, S. B. (1970). Some aspects of host-parasite relationships in mesopelagic fishes. *In* "A symposium on diseases of fishes and shellfishes" (S. F. Snieszko, ed.), pp. 41–56. *Special Publication. American Fisheries Society,* No. 5.

Coombs, R. R. A. and Gell, P. G. H. (1968). Classification of allergic reactions responsible for clinical hypersensitivity and disease. *In* "Clinical Aspects of Immunology" 2nd Edition. (P. G. H. Gell and R. R. A. Coombs, eds), pp. 575–596. Blackwell Scientific Publications, Oxford, London, Edinburgh and Melbourne.

Cowan, D. F. (1967). Helminth parasites of the pilot whale *Globicephala melaena* (Traill 1809). *Journal of Parasitology* **53**, 166–167.

Cushing, D. H. (1975). "Marine Ecology and Fisheries" Cambridge University Press, Cambridge, London, New York and Melbourne.

Dailey, M. D. and Brownell, R. L., Jr. (1972). A checklist of marine mammal parasites. *In* "Mammals of the Sea. Biology and Medicine" (S. H. Ridgway, ed.), pp. 528–589. Charles C. Thomas, Springfield, Illinois, USA.

Davey, J. T. (1971a). A revision of the genus *Anisakis* Dujardin, 1845 (Nematoda: Ascaridata). *Journal of Helminthology* **45**, 51–72.

Davey, J. T. (1971b). A case of *Anisakis* sp. infection in a brown bear from Alaska. (Demonstration.) *Transactions of the Royal Society of Tropical Medicine and Hygiene* **65**, 433–434.

Davey, J. T. (1972a). The incidence of *Anisakis* sp. larvae (Nematoda: Ascaridata) in the commercially exploited stocks of herring (*Clupea harengus* L., 1758) (Pisces: Clupeidae) in British and adjacent waters. *Journal of Fish Biology* **4**, 535–554.

Davey, J. T. (1972b). The public health aspects of a larval roundworm from the herring. *Community Health* **4**, 104–106.

Davey, K. G. (1976). Hormones in nematodes. *In* "The Organization of Nematodes" (N. A. Croll, ed.), pp. 273–291. Academic Press, New York and London.

Delyamure, S. L., Kurochkin, Y. V. and Skryabin, A. S. (1964). (Contributions to

the helminth fauna of the Caspian seal (*Phoca caspica* Gm.).) *Trudy Astrakhanskogo Zapovednika* No. 9, 105–118. (In Russian.)

Doby, J. M., Le Masson, J. M. and Babin, P. (1975). Granulome éosinophilique du colon par larve de nématode Anisakidae. *Bulletin de la Société de pathologie exotique* **67**, 522–536.

Dollffus, R. P. (1953). Apercu général sur l'histoire naturelle des parasites animaux de la morue atlanto-arctique *Gadus callarias* L. (= *morhua* L.). *Encyclopédie Biologique* **43**, 1–423.

Dollfus, R. P. (1970). Les vers nématodes du hareng. *Comptes Rendus du Quatre-Vingt-Quatorzième Congrès National des Sociétés Savantes.* Pau, 1969, Vol. 3, pp. 351–426.

Eisen, H. N. (1974). Immunology. An introduction to molecular and cellular principles of the immune responses. *In* "Microbiology" 2nd edition (Davis *et al.*, eds), pp. 352–624. Harper and Row, Maryland, USA.

Ergens, R., Gussev, V. A., Izyumova, N. A. and Molnár, K. (1975). Parasite fauna of fishes of the Tisa River Basin. *Rozpravy Československé Akademie Věd: Řada Matematickych a Prírodních Věd* **85**, 3–117.

Euzéby, J. (1973). Un type de zoonose helminthique méconnu: les ascaridoses larvaires de l'homme d'origine pisciaire: larva migrans éosinophilique du tractus gastro-intestinal ("Maladie du ver du hareng"). *Revue médicale et Vétérinaire* **124**, 43–68.

Evans, P. G. H. (1976). An analysis of sightings of Cetacea in British waters. *Mammal Review* **6**, 5–14.

Fain, A., van Roy, M. and Fontenelle, E. (1969). Un phlegmon du jéjunum produit par une larve d'Anisakis. *Louvain Médecine* **88**, 579–587.

Florkin, M. and Scheer, B. T. (Eds) (1969). "Chemical Zoology. Vol. III. Echinodermata, Nematoda and Acanthocephala" Academic Press, New York and London.

Gembardt, C., Hildebrand, B. and Göltenboth, R. (1971). Durch *Anisakis*-Befall hervorgerufene Pylorusstenose bei einem See-Elefanten (*Mirounga leonina*). *Berliner und Münchener tierärztliche Wochenschrift* **84**, 193–195.

Gibson, D. I. (1970). Aspects of the development of "herringworm" (*Anisakis* sp. larva) in experimentally infected rats. *Nytt Magasin for Zoologi* **18**, 175–187.

Gibson, D. I. (1972). Flounder parasites as biological tags. *Journal of Fish Biology* **4**, 1–9.

Gibson, D. I. (1973). The genus *Pseudanisakis* Layman and Borovkova, 1926 (Nematoda: Ascaridida). *Journal of Natural History* **7**, 319–340.

Gibson, D. I. (1974). The type-species of the genus *Pseudanisakis* Layman and Borovkova, 1926 (Nematoda). Z. N. (S.) 2020. *Bulletin of Zoological Nomenclature* **30**, 182–184.

Gibson, D. I. and Taylor, A. L. (1976). A comment upon the taxonomic significance and function of the "excretory system" of ascaridoid nematodes. (Abstract.) *Parasitology* **73**, v.

Grabda, J. (1973). (Studies on the survival of *Anisakis simplex* larvae.) (IV Sesja Naukowa Medcyny Morskiej Szozecin 18–19 V. 1973 R.) *Annales Academiae Medicae Stetinensis* (*Roczniki Pomorskiej Akademii Medycznej w Szczecinie*) Suppl. **10**, 47–52. (In Polish.)

Grabda, J. (1974). The dynamics of the nematode larvae, *Anisakis simplex* (Rud.) invasion in the south-western Baltic herring (*Clupea harengus* L.) *Acta Ichthyologica et Piscatoria* **4**, 3–21.

Grabda, J. (1976). Studies on the life cycle and morphogenesis of *Anisakis simplex*

(Rudolmaphi, 1809) (Netoda: Anisakidae) cultured in vitro. *Acta Ichthylogica et Piscatoria* **6**, 119–141.

Grainger, J. N. R. (1959). The identity of the larval nematodes found in the body muscles of the cod (*Gadus callarias* L.). *Parasitology* **49**, 121–131.

Griner, L. A. (1974). Some diseases of zoo animals. *In* "Advances in Veterinary Science and Comparative Medicine" (C. A. Brandly and C. E. Cornelius, eds), 18, Vol. pp. 251–271. Academic Press, New York and London.

Guiart, J. (1938). Étude parasitologique et épidémiologique de quelques poissons de mer. *Bulletin de l'Institut océanographique de Monaco* No. 755, 15 pp.

Gustafson, P. V. (1953). The effect of freezing on encysted *Anisakis* larvae. *Journal of Parasitology* **39**, 585–588.

Halvorsen, O. and Williams, H. H. (1968). Studies on the helminth fauna of Norway. IX. *Gyrocotyle* (Platyhelminthes) in *Chimaera monstrosa* from Oslo Fjord, with emphasis on its mode of attachment and a regulation in the degree of infection. *Nytt Magasin for Zoologi* **15**, 130–142.

Hamajima, F., Nishihara, S. and Yasuhiro, C. (1969). Glycolytic and oxidative metabolism in the larvae of a nematode, *Anisakis* sp. *Japanese Journal of Parasitology* **18**, 196–201.

Hartwich, G. (1974). Keys to genera of the Ascaridoidea. *In* "CIH Keys to the Nematode Parasites of Vertebrates" (R. C. Anderson, A. Chabaud and S. Willmott, eds) No. 2, 15 pp. Commonwealth Agricultural Bureaux, Farnham Royal, Bucks, England.

Hashiguchi, Y. and Takei, T. (1975). (Effects of various temperatures on the infectivity of *Anisakis* larvae to albino rat.) *Japanese Journal of Parasitology* **24**, 34–40. (In Japanese.)

Hennig, H. F. K. O. (1974). The effect of a larval *Anisakis* (Nematoda: Ascaroidea) on the South West African anchovy, *Engraulis capensis*. *Journal du Conseil* **35**, 185–188.

Hewitt, G. C. and Hine, P. M. (1972). Checklist of parasites of New Zealand fishes and of their hosts. *New Zealand Journal of Marine and Freshwater Research* **6**, 69–114.

Hirao, Y. and Yamaguchi, T. (1964). (Studies of larva migrans (18); experiments on infection of various animals with *Anisakis*-type larvae.) *Japanese Journal of Parasitology* **14**, 620–621. (In Japanese.)

Hislop, J. R. G. and MacKenzie, K. (1976). Population studies of the whiting *Merlangius merlangus* (L.) of the northern North Sea. *Journal du Conseil* **37**, 98–111.

Hitchcock, D. J. (1950). Parasitological study on the Eskimos in the Bethal area of Alaska. *Journal of Parasitology* **36**, 232–234.

Hoeppli, R. (1933). On histolytic changes and extraintestinal digestion in parasite infections. *Lingnan Science Journal* **12**, 1–11 (Suppl.).

Hsieh, H. C. and Chen, E. R. (1970). *Anisakis* larvae in the stomach wall of the Taiwan monkey (*Macaca cyclopis*). (Demonstration.) *Southeast Asian Journal of Tropical Medicine and Public Health* **1**, 567–568.

Hsü, H. F. (1933). Study on the oesophageal glands of parasitic Nematoda superfamily Ascaroidea. *Chinese Medical Journal* **47**, 1247–1288.

Huizinga, H. W. (1966). Studies on the life cycle and development of *Contracaecum spiculigerum* (Rudolphi, 1809) (Ascaroidea: Heterocheilidae) from marine piscivorous birds. *Journal of the Elisha Mitchell Scientific Society* **82**, 181–195.

Huizinga, H. W. (1967). The life cycle of *Contracaecum multipapillatum* (von Drasche, 1882) Lucker, 1941 (Nematoda: Heterocheilidae). *Journal of Parasitology* **53**, 368–375.

Hyman, L. H. (1951). "The Invertebrates: Acanthocephala, Aschelminthes, and Entoprocta. The Pseudocoelomate Bilateria. Vol. III." McGraw-Hill Book Co., Inc., New York, Toronto and London.

Inglis, W. G. (1954). Allometric growth in the Nematoda. *Nature, London,* **173,** 957.

Inglis, W. G. (1964a). The functional and developmental significance of the cephalic septum in the Ascaridoidea (Nematoda). *Proceedings of the Linnean Society of London* **176,** 23–36.

Inglis, W. G. (1964b). The comparative anatomy of the ascaridoid cuticle (Nematoda). *Bulletin de la Société zoologique de France* **89,** 317–338.

Ishikura, H., Hayasaka, H. and Kikuchi, Y. (1967). Acute regional ileitis at Iwanai in Hokkaido with special reference to intestinal anisakiasis. *Sapporo Medical Journal* **32,** 183–196.

Jackson, G. J. (1975). The "new disease" status of human anisakiasis and North American cases: a review. *Journal of Milk and Food Technology* **38,** 769–773.

Jackson, G. J., Bier, J. W. and Payne, W. L. (1976). Experimental anisakiasis in pigs: course of infection with larval *Anisakis* sp. and *Phocanema* sp. nematodes from fishes. (Abstract.) *Transactions of the American Microscopical Society* **95,** 264.

Jacobsen, K. B. and Berland, B. (1969). (Fish nematodes as a cause of acute and chronic gastroenteritis with tissue eosinophilia.) *Nordisk Medicin* **82,** 1104–1111. (In Norwegian.)

Jueco, N. L., Bobis, T. A. and Ramirez, L. M. (1971). Seasonal prevalence and density of *Anisakis* larvae in fishes (galunggong) sold in public markets in Manila. *Journal of the Philippine Medical Association* **47,** 467–476.

Kabata, Z. (1963). Parasites as biological tags. *ICNAF Special Publication,* No. 4, 31–37.

Kagei, N. (1969). (Life-history of nematodes of the genus *Anisakis*.) *Saishin-Igaku* (Advances in Medical Sciences), **24,** 389–400. (In Japanese.)

Kagei, N. (1970). List of the larvae of *Anisakis* spp. recorded from marine fish and squids caught off the Japan and its offshore islands. *Bulletin of the Institute of Public Health, Tokyo* **19,** 76–85.

Kagei, N. (1974). (Studies on anisakid nematodes (Anisakinae). IV. Survey of *Anisakis* larvae in marine crustacea.) *Bulletin of the Institute of Public Health, Tokyo* **23,** 65–71. (In Japanese.)

Kagei, N. and Kureha, K. (1970). (Studies on anisakid Nematoda (Anisakinae) I. Survey of *Anisakis* sp. in marine mammals collected in the Antarctic Ocean.) *Bulletin of the Institute of Public Health, Tokyo* **19,** 193–196. (In Japanese.)

Kagei, N., Oshima, T., Kobayashi, A., Kumada, M., Koyama, T., Komiya, Y. and Takemura, A. (1967). (Survey of *Anisakis* spp. (Anisakinae, Nematoda) in marine mammals on the coast of Japan.) *Japanese Journal of Parasitology* **16,** 427–435. (In Japanese.)

Kahl, W. (1938). Nematoden in Seefischen. II. Erhebungen über den Befall von Seefischen mit Larven von *Anacanthocheilus rotundatus* (Rudolphi) und die durch diese Larven hervorgerufenen Reaktionen des Wirtsgewebes. *Zeitschrift für Parasitenkunde* **10,** 513–534.

Kane, M. B. (1966). Parasites of Irish fishes. *Scientific Proceedings of the Royal Dublin Society, Series B* **1,** 205–221.

Kawada, S. (1968). (Studies on the prevention of anisakiasis. Resistance of *Anisakis* larvae in various media.) *Journal of Osaka Medical College* **26,** 224–244. (In Japanese.)

Kawai, Y. (1968). (Free fatty acids of *Ascaris lumbricoides suum* and *Anisakis* sp. larvae.) *Japanese Journal of Parasitology* **17**, 182–186. (In Japanese.)

Kennedy, C. R. (1974). A checklist of British and Irish freshwater fish parasites with notes on their distribution. *Journal of Fish Biology* **6**, 613–644.

Khalil, L. F. (1969). Larval nematodes in the herring (*Clupea harengus*) from British coastal waters and adjacent territories. *Journal of the Marine Biological Association of the United Kingdom* **49**, 641–659.

Kikuchi, S., Hayashi, S. and Nakajima, M. (1967). (Studies on anisakiasis in dolphins.) *Japanese Journal of Parasitology* **16**, 156–166. (In Japanese.)

Kilambi, R. V. and DeLacy, A. C. (1967). Heterogeneity of surf smelt, *Hypomesus pretiosus* (Girard), in the state of Washington, as judged by incidence of larval *Anisakis* (Nematoda). *Journal of the Fisheries Research Board of Canada* **24**, 629–633.

Kim, C. H., Chung, B. S., Moon, Y. I. and Chun, S. H. (1971). (A case report on human infection with *Anisakis* sp. in Korea.) *Korean Journal of Parasitology* **9**, 39–45. (In Korean.)

Kitayama, H., Ohbayashi, M., Satoh, H. and Kitamura, Y. (1967). (Studies on parasitic granuloma in the dog.) *Japanese Journal of Parasitology* **16**, 28–35. (In Japanese.)

Klumov, S. K. (1963). (Food and helminth fauna of whalebone whales (Mystacoceti) in the main whaling regions of the world ocean.) *Trudȳ Instituta okeanologii. Akademiya nauk SSSR* **71**, 94–194. (In Russian.)

Kobayashi, A., Koyama, T., Kumada, M., Komiya, Y., Oshima, T., Kagei, N., Ishii, T. and Machida, M. (1966). (A survey of marine fishes and squids for the presence of Anisakinae larvae.) *Japanese Journal of Parasitology* **15**, 348–349. (In Japanese.)

Kobayashi, A., Koyama, T., Kumada, M., Oshima, T. and Kagei, N. (1968a). (On the development of *Anisakis* (*A. simplex*?) eggs.) *Japanese Journal of Parasitology* **17**, 583. (In Japanese.)

Kobayashi, A., Kumada, M., Ishizaki, T., Suguro, T. and Koito, K. (1968b). (Skin tests with somatic and ES (excretions and secretions) antigens from *Anisakis* larvae. I. Survey of normal populations on the skin sensitivity to the different antigens.) *Japanese Journal of Parasitology* **17**, 407–413. (In Japanese.)

Kobayashi, A., Kumada, M., Ishizaki, T., Suguro, T. and Koito, K. (1968c). (Skin tests with somatic and ES (excretions and secretions) antigens from *Anisakis* larvae. II. The difference of antigenicity between the two antigens.) *Japanese Journal of Parasitology* **17**, 414–418. (In Japanese.)

Kobayashi, A., Kumada, M. and Ishizaki, T. (1972). Evaluation of somatic and "ES" antigens causing immunological injury of mast cells in mice infected with *Anisakis* larvae. *Japanese Journal of Medical Science and Biology* **25**, 335–344.

Kobayashi, A., Watanabe, N. and Endo, T. (1974). Suppression of immunological mast cell degranulation to bovine serum albumin in mice following *Anisakis* infection. (Abstract.) *Proceedings of the 3rd International Congress of Parasitology Munich, Aug. 25–31, 1974*, **2**, 1075–1076. FACTA Publication, Vienna, Austria.

Konovalov, S. M. (1967). (On the use of parasitological data in the differentiation of local stocks of Pacific salmon.) *Parazitologicheskiĭ Sbornik* **23**, 236–249. (In Russian.)

Koyama, T., Yasuraoka, K. and Machida, M. (1967). (Studies on the *in vitro* axenic development of *Anisakis* larvae. (II).) *Japanese Journal of Parasitology* **16**, 291. (In Japanese.)

Koyama, T., Kobayashi, A., Kumada, M., Komiya, Y., Oshima, T., Kagei, N.,

Ishii, T. and Machida, M. (1969). (Morphological and taxonomical studies on Anisakidae larvae found in marine fishes and squids.) *Japanese Journal of Parasitology* **18**, 466–487. (In Japanese.)

Kozima, K., Oyanagi, T. and Shiraki, K. (1966). (Pathology of anisakiasis.) *Nippon Rinsho* **24**, 2314–2323. (In Japanese.)

Kreis, H. A. (1958). Parasitic Nematoda. *Zoology of Iceland* **11** (15b), 24 pp.

Kuipers, F. C. (1962). Eosinofiele flegmone van de dunne darm. *Tijdschrift voor gastroenterologie* **5**, 320–327.

Kuipers, F. C. (1964). Pathogenese van de haringwormflegmone bij de mens. *Nederlands Tijdschrift voor Geneeskunde* **108**, 304–305.

Kuipers, F. C., Kampelmacher, E. H. and Steenbergen, F. (1963). Onderzoekingen over haringwormziekte bij konijnen. *Nederlands Tijdschrift voor Geneeskunde* **107**, 990–995.

Kurochkin, Y. V. (1972). (Parasite fauna of *Todarodes pacificus.*) *In* "Parazity vodnykh bespozvonochnykh zhivotnykh. I Vsesoyuznyi Simposium" 'L'vov, USSR: Izdatel'stvo L'vovskogo Universiteta, pp. 53–54. (In Russian.)

Kurochkin, Y. V. (1975). Parasites of the Caspian seal *Pusa caspica. Rapport et procès-verbaux des réunions. Conseil international pour l'exploration de la mer* 169, 363–365.

Kurochkin, Y. V. and Leont'eva, V. G. (1970). (Medical significance and distribution in marine fish of anisakid larvae.) Voprosy morskoi parazitologii (All-Union symposium (1st) on parasites and diseases of marine animals, Sevastopol, 1970). *Kiev: Izdatel'stvo "Naukova Dumka"*, pp. 56–59. (In Russian.)

Lasker, R. and Theilacker, G. H. (1965). Maintenance of euphausiid shrimps in the laboratory. *Limnology and Oceanography* **10**, 287–288.

Lee, D. L. and Atkinson, H. J. (1976). "The Physiology of Nematodes" 2nd edition. Macmillan Press Ltd., London and Basingstoke.

Lee, H. F., Chen, I. L. and Lin, R. P. (1973). Ultrastructure of the excretory system of *Anisakis* larva (Nematoda: Anisakidae). *Journal of Parasitology* **59**, 289–298.

Lee, W. H. and Chyu, I. (1970). (Experiments on the resistance and infectivity of *Anisakis* larvae.) *Journal of the Catholic Medical College* **18**, 229–237. (In Korean.)

Lester, R. J. G. (1977). An estmiate of the mortality in a population of *Perca flavescens* owing to the trematode *Diplostomum adamsi. Canadian Journal of Zoology* **55**, 288–292.

Longhurst, A., Colebrook, M., Gulland, J., Le Brasseur, R., Lorenzen, C. and Smith, P. (1972). The instability of ocean populations. *New Scientist* **54** (798), 500–502.

McClelland, G. and Ronald, K. (1974a). In vitro development of *Terranova decipiens* (Nematoda) (Krabbe, 1878). *Canadian Journal of Zoology* **52**, 471–479.

McClelland, G. and Ronald, K. (1974b). In vitro development of the nematode *Contracaecum osculatum* Rudolphi 1802 (Nematoda: Anisakinae). *Canadian Journal of Zoology* **52**, 847–855.

MacKenzie, K. and Wootten, R. (1973). Parasites as biological tags for whiting populations in the northern North Sea. *ICES Demersal Fish (Northern) Committee*, C. M. 1973/F: 48, 7 pp.

McLaren, D. J. (1976). Nematode sense organs. *Advances in Parasitology* **14**, 195–265.

Margolis, L. (1970). Nematode diseases of marine fishes. *In* "A symposium on diseases of fishes and shellfishes" (S. F. Snieszko, ed.), pp. 190–208. *Special Publication. American Fisheries Society*, No. 5.

Matthews, B. E. (1977). The passage of larval helminths through tissue barriers. *In* "Parasite Invasion" (A. E. R. Taylor and R. Muller, eds), pp. 93–119. 15th Symposium of the British Society for Parasitology. Blackwell Scientific Publications, Oxford and Edinburgh.

Mauchline, J. and Fisher, L. R. (1969). The biology of euphausiids. *Advances in Marine Biology* **7**, ix + 454 pp.

Migaki, G., Van Dyke, D. and Hubbard, R. C. (1971). Some histopathological lesions caused by helminths in marine mammals. *Journal of Wildlife Diseases* **7**, 281–289.

Mikhailova, I. G., Prazdnikov, E. V. and Prusevich, T. O. (1964). (Morphological changes in the fish tissue around the larvae of some parasitic worms.) *Trudy Murmanskogo biologicheskogo instituta* **5**, 251–264. (In Russian.)

Mokhayer, B. (1974). (Ecological character of the parasitic diseases of Iranian sturgeons.) *Journal of the Veterinary Faculty, University of Tehran* **30**, 38–48. (In Persian.)

Morishita, K. and Nishimura, T. (1965). (Studies on *Anisakis* larva (2). *Anisakis* larva from human buccal mucosa.) *Japanese Journal of Parasitology* **14**, 54. (In Japanese.)

Mueller, J. F. (1927). The excretory system of *Anisakis simplex*. *Zeitschrift für Zellforschung und mikroskopische Anatomie* **5**, 495–504.

Murie, J. (1868). On the morbid appearance observed in the walrus lately living in the Society's gardens. With a description of a new species of *Ascaris* found in the stomach; by Dr Baird F.L.S. *Proceedings of the Zoological Society of London* **5**, 67–71.

Myers, B. J. (1960). On the morphology and life history of *Phocanema decipiens* (Krabbe, 1878) Myers, 1959 (Nematoda: Anisakidae). *Canadian Journal of Zoology* **38**, 331–344.

Myers, B. J. (1963). The migration of *Anisakis*-type larvae in experimental animals. *Canadian Journal of Zoology* **41**, 147–148.

Myers, B. J. (1975). The nematodes that cause anisakiasis. *Journal of Milk and Food Technology* **38**, 774–782.

Myers, B. J. (1976). Research then and now on the Anisakidae nematodes. *Transactions of the American Microscopical Society* **95**, 137–142.

Nagase, K. (1968a). (Studies on *Anisakis* (1). On LDH isozymes.) *Japanese Journal of Parasitology* **17**, 86–89. (In Japanese.)

Nagase, K. (1968b). (Studies of *Anisakis* (2). Behaviour of *Anisakis* larvae in rats.) *Japanese Journal of Parasitology* **17**, 368–375. (In Japanese.)

Nakamura, K., Toyokawa, O. and Aizawa, M. (1974). (Cellular immunity in experimental anisakiasis.) (Abstract.) *Japanese Journal of Parasitology* **23**, (2, Suppl.), 67–68. (In Japanese.)

Noble, E. R. (1973). Parasites and fishes in a deep-sea environment. *Advances in Marine Biology* **11**, 121–195.

Noble, E. R. and Collard, S. B. (1970). The parasites of midwater fishes. *In* "A symposium on diseases of fishes and shellfishes" (S. F. Snieszko, ed.), pp. 57–68. *Special Publication. American Fisheries Society*, No. 5.

Norris, D. E. and Overstreet, R. M. (1976). The public health implications of larval *Thynnascaris* nematodes from shellfish. *Journal of Milk and Food Technology* **39**, 47–54.

Novotny, A. J. and Uzmann, J. R. (1960). A statistical analysis of the distribution of a larval nematode (*Anisakis* sp.) in the musculature of chum salmon (*Oncorhynchus keta*—Walbaum). *Experimental Parasitology* **10**, 245–262.

Nyman, O. L. and Pippy, J. H. C. (1972). Differences in Atlantic salmon, *Salmo*

salar, from North America and Europe. *Journal of the Fisheries Research Board of Canada* **29**, 179–185.

Odening, K. (1976). Conception and terminology of hosts in parasitology. *Advances in Parasitology* **14**, 1–93.

Oishi, K., Oka, S. and Josho, S. (1969). (An introduction to food hygiene of the *Anisakis* larva.) Hakodate Food Science Research Society (Pamphlet, 113 pp.), Hokkaido, Japan. (In Japanese.)

Oishi, K., Oka, S. and Hiraoki, M. (1971). (Food hygienic studies on *Anisakis* larvae. I. Numerical detective method of the larvae in the muscle and viscera of sea animals.) *Bulletin of the Japanese Society of Scientific Fisheries* **37**, 186–191. (In Japanese.)

Oishi, K., Oka, S. and Hiraoki, M. (1972a). (Food hygienic studies on *Anisakis* larvae. II. Free and combined amino-acid composition of *Anisakis* larvae (the 3rd stage) from Alaska pollack.) *Bulletin of the Japanese Society of Scientific Fisheries* **38**, 69–72. (In Japanese.)

Oishi, K., Oka, S. and Hiraoki, M. (1972b). (Food hygienic studies on *Anisakis* larvae. III. Effect of gamma irradiation on *Anisakis* larvae in saline solution.) *Bulletin of the Japanese Society of Scientific Fisheries* **38**, 133–136. (In Japanese.)

Oishi, K., Mori, K. and Nishiura, Y. (1974). (Food hygiene studies on Anisakinae larvae. V. Effects of some spice essential oils and food preservatives on the mortality of Anisakinae larvae.) *Bulletin of the Japanese Society of Scientific Fisheries* **40**, 1241–1250. (In Japanese.)

Okumura, T. (1967). (Experimental studies on anisakiasis.) *Journal of the Osaka City Medical Center* **16**, 465–499. (In Japanese.)

Okuno, Y. (1968). (Amino-acids of hydrolysed tissues of pig *Ascaris* and *Anisakis* sp. larvae. I. Analysis by thin-layer chromatography.) *Japanese Journal of Parasitology* **17**, 199–207. (In Japanese.)

Okuno, Y. (1969). (Amino acids in *Ascaris suum* and *Anisakis* sp. larva. Analysis by thin-layer chromatography and amino acid autoanalyzer.) *Japanese Journal of Parasitology* **18**, 77–86. (In Japanese.)

Oshima, T. (1969). (A study on the first host of *Anisakis*.) *Saishin-Igaku* (Advances in Medical Sciences), **24**, 401–404. (In Japanese.)

Oshima, T. (1972). *Anisakis* and anisakiasis in Japan and adjacent area. *In* "Progress of Medical Parasitology in Japan" (K. Morishita, Y. Komiya and H. Matsubayashi, eds), Vol. IV, pp. 301–393. Meguro Parasitological Museum, Tokyo.

Oshima, T., Kobayashi, A., Kumada, T., Koyama, T., Kagei, N. and Nemoto, T. (1968). (Experimental infection with second-stage larvae of *Anisakis* sp. of *Euphausia similis* and *Euphausia pacifica*.) *Japanese Journal of Parasitology* **17**, 585. (In Japanese.)

Oshima, T., Shimazu, T., Koyama, H. and Akahane, H. (1969). (On the larvae of the genus *Anisakis* (Nematoda: Anisakinae) from euphausiids.) *Japanese Journal of Parasitology* **18**, 241–248. (In Japanese.)

Oshmarin, P. G. and Demshin, N. I. (1972). (The helminths of domestic and some wild animals in Vietnam.) *Trudȳ Biologo-Pochyennogo Instituta; Dal'nevostochnyĭ Nauchnyĭ Tsentr AN SSSR (Issledovaniya po faune, sistematike i biokhimii gel'mintov Dal'nego Vostoka)*, **11**, 5–115. (In Russian.)

Otsuru, M., Hatsukano, T., Oyanagi, T. and Kenmotsu, M. (1965). (The visceral migrans of gastro-intestinal tract and its vicinity caused by some larval nematode.) *Japanese Journal of Parasitology* **14**, 542–555. (In Japanese.)

Otsuru, M., Shiraki, T., Hatsukano, T. and Kenmotsu, M. (1968). (Morphological

observations and infection experiments on Anisakinae larvae of fishes in Hokkaido coastal waters.) *Japanese Journal of Parasitology* **17**, 267. (In Japanese.)

Otsuru, M., Shiraki, T. and Kenmotsu, M. (1969). (The classification, morphology and experimental infection of Anisakinae larvae found in marine fishes in the sea surrounding the North of Japan.) *Japanese Journal of Parasitology* **18**, 417–418. (In Japanese.)

Oyanagi, T. (1967). (Experimental studies on the visceral migrans of gastrointestinal walls due to *Anisakis* larvae.) *Japanese Journal of Parasitology* **16**, 470–493. (In Japanese.)

Parsons, L. S. and Hodder, V. M. (1971). Variation in the incidence of larval nematodes in herring from Canadian Atlantic waters. *Research Bulletin. International Commission for the Northwest Atlantic Fisheries* No. **8**, 5–14.

Parukhin, A. M. (1971). (Nematodes of fish in the Red Sea and Indian Ocean.) *Biologiya Morya, Kiev*, No. **23**, 177–193. (In Russian.)

Petrushevski, G. K. and Kogteva, E. P. (1954). (Effect of parasitic diseases on condition of fish.) *Zoologicheskiĭ Zhurnal* **33**, 395–405. (In Russian.)

Petter, A. J. (1972). Redescription of *Anisakis insignis* Diesing (Ascaridoïdea), parasite of the Amazon dolphin, *Inia geoffrensis*. *In* "Investigations on Cetacea" (G. Pilleri, ed.), Vol. IV, pp. 93–99. Brain Anatomy Institute, University of Berne, Switzerland.

Pinkus, G. S., Coolidge, C. and Little, M. D. (1975). Intestinal anisakiasis. First case report from North America. *American Journal of Medicine* **59**, 114–120.

Pippy, J. H. C. (1969). Preliminary report on parasites as biological tags in Atlantic salmon (*Salmo salar*). I. Investigations 1966 to 1968. *Technical Report. Fisheries Research Board of Canada*, No. **134**, 44 pp.

Pippy, J. H. C. and Van Banning, P. (1975). Identification of *Anisakis* larva (1) as *Anisakis simplex* (Rudolphi, 1809 det. Krabbe 1878) (Nematoda: Ascaridata). *Journal of the Fisheries Research Board of Canada* **32**, 29–32.

Polyanski, Y. I. (1966). "Parasites of the fish of the Barents Sea." Israel Program for Scientific Translations, Jerusalem, 158 pp. (Translated from Russian.)

Porath, J., Axén, R. and Ernback, S. (1967). Chemical coupling of proteins to agarose. *Nature, London* **215**, 1491–1492.

Power, H. E. (1961). Slicing of fillets as an aid in detection and removal of codworms from Atlantic cod fillets. *Journal of the Fisheries Research Board of Canada* **18**, 137–140.

Poynter, D. (1966). Some tissue reactions to the nematode parasites of animals. *Advances in Parasitology* **4**, 321–383.

Priebe, K., Jendrusch, H. and Haustedt, U. (1973). Problematik und Experimentaluntersuchungen zum Erlöschen der Einbohrpotenz von *Anisakis*-Larven des Herings bei der Herstellung von Kaltmarinaden. *Archiv für Lebensmittelhygiene* **24**, 217–222.

Prusevich, T. O. (1964). (On the formation of capsules around larvae of *Anisakis* sp. in the tissues of the shorthorn sculpin *Myoxocephalus scorpius*.) *Trudȳ Murmanskogo Biologicheskogo Instituta* **5**, 265–273. (In Russian.)

Punt, A. (1941). Recherches sur quelques nématodes parasites de poissons de la Mer du Nord. *Mémoires du Musée r. d'histoire naturelle de Belgique* No. **98**, 110 pp.

Rae, B. B. (1963). The incidence of larvae of *Porrocaecum decipiens* in the flesh of cod. Marine Research. Department of Agriculture and Fisheries for Scotland, 1963, No. 2, 28 pp.

Rae, B. B. (1965). The food of the common porpoise (*Phocaena phocaena*). *Journal of Zoology* **146**, 114–122.

Rae, B. B. (1972). A review of the cod-worm problem in the North Sea and in Western Scottish waters 1958–1970. Marine Research. Department of Agriculture and Fisheries for Scotland, 1972, No. 2, 24 pp.

Reimer, L. W., Berger, C., Heuer, B., Lainka, H., Rosenthal, I. and Scharnweber, I. (1971). (On the distribution of larvae of helminths in plankton animals of the North Sea.) *Parazitologiya* **5**, 542–550. (In Russian.)

Remotti, E. (1933). Ancora sull' *Ascaris capsularia* Rud. *Bollettino dei Musei e laboratorii di zoologia e di anatomia comparata della R. Università di Genova* (68), 1–15.

Ridgway, S. H. (1972). Homeostasis in the aquatic environment. *In* "Mammals of the Sea. Biology and Medicine" (S. H. Ridgway, ed.), pp. 590–747. Charles C. Thomas, Springfield, Illinois, USA.

Riley, J. (1972). The pathology of *Anisakis* nematode infections of the fulmar *Fulmarus glacialis*. *Ibis* **114**, 102–104.

Rodenburg, W. and Wielinga, W. J. (1960). Eosinofiele flegmone van de dunne darm, veroorzaakt door een worm. *Nederlands Tijdschrift voor Geneeskunde* **104**, 417–421.

Roitt, I. M. (1971). "Essential Immunology" Blackwell Scientific Publications, Oxford, London, Edinburgh and Melbourne.

Ronald, K. (1960). The effects of physical stimuli on the larval stage of *Terranova decipiens* (Krabbe, 1878) (Nematoda: Anisakidae). I. Temperature. *Canadian Journal of Zoology* **38**, 623–642.

Ronald, K. (1962). The effects of physical stimuli on the larvae of *Terranova decipiens*. II. Relative humidity, pressure, and gases. *Canadian Journal of Zoology* **40**, 1223–1227.

Ronald, K. (1963). The effects of physical stimuli on the larval stage of *Terranova decipiens*. III. Electromagnetic spectrum and galvanotaxis. *Canadian Journal of Zoology* **41**, 197–217.

Ruitenberg, E. J. (1970). Anisakiasis. Pathogenesis, serodiagnosis and prevention. Thesis: Rijksuniversiteit, Utrecht, 138 pp.

Ruitenberg, E. J. (1971). Experimental anisakiasis in rabbits. *In* "Pathology of Parasitic Diseases" (S. M. Gaafar, ed.), pp. 359–366. Purdue University Studies, Lafayette, Indiana.

Ruitenberg, E. J. (1972). Acid phosphatases in the intestinal cells of two nematode larvae: *Anisakis* sp. and *Trichinella spiralis*. *In* "Comparative Biochemistry of Parasites" (H. Van den Bossche, ed.), pp. 283–295. Academic Press, New York and London.

Ruitenberg, E. J. and Loendersloot, H. J. (1971a). Enzymhistochemische onderzoek van *Anisakis* sp. larven. *Tidjschrift voor Diergeneeskunde* **96**, 247–260.

Ruitenberg, E. J. and Loendersloot, H. J. (1971b). Histochemical properties of the excretory organ of *Anisakis* sp. larva. *Journal of Parasitology* **57**, 1149–1150.

Ruitenberg, E. J., Berkvens, J. M. and Duyzings, M. J. M. (1971). Experimental *Anisakis marina* infections in rabbits. *Journal of Comparative Pathology* **81**, 157–163.

Saeki, H., Kikuchi, K., Nishino, C., Iwano, H., Shimodan, M. and Hayasaka, H. (1973). (Studies on cell-mediated immunity in experimental anisakiasis.) (Abstract.) *Japanese Journal of Parasitology* **22** (2, Suppl.), 60. (In Japanese.)

Saeki, H., Hayasaka, H. and Kikuchi, K. (1975). (Studies on delayed hypersensitivity in experimental anisakiasis.) (Abstract.) *Japanese Journal of Parasitology* **24** (2, Suppl.), 63–64. (In Japanese.)

Sato, Y., Suzuki, T., Yamashita, T., Shiraki, T., Sekikawa, H. and Otsuru, M. (1973). (Indirect fluorescent antibody test for diagnosis of anisakiasis. 1. Preparation of antigens.) *Japanese Journal of Parasitology* **22**, 249–258. (In Japanese.)

Sato, Y., Suzuki, T., Shiraki, T., Yamashita, T. and Otsuru, M. (1974). (Studies on the immunological diagnosis of anisakiasis. IV. Antigenic specificity of haemoglobin of *Anisakis* larvae and further purification of antigen using immunoadsorbent.) *Japanese Journal of Parasitology* **23**, 356–365. (In Japanese.)

Sato, Y., Yamashita, T., Otsuru, M., Suzuki, T., Asaishi, K. and Nishino, C. (1975). (Studies on the aetiology of anisakiasis. 1. The anaphylactic reaction of digestive tract to the worm extracts.) *Japnaese Journal of Parasitology* **24**, 192–202. (In Japanese.)

Schulz, H. P. (1974). *Anisakis* larvae from the herring: *in vitro* development and morphology. (Abstract.) *Proceedings of the 3rd International Congress of Parasitology, Munich, Aug. 25–31, 1974*, **3**, 1627. FACTA Publication, Vienna, Austria.

Shimazu, T. and Oshima, T. (1972). Some larval nematodes from euphausiid crustaceans. *In* "Biological Oceanography of the Northern North Pacific Ocean dedicated to Shigeru Motoda" (A. Y. Takenouti *et al.*, eds), pp. 403–409. Idemitsu Shoten, Tokyo, Japan.

Shiraki, T. (1974). Larval nematodes of family Anisakidae (Nematoda) in the Northern Sea of Japan—as a causative agent of eosinophilic phlegmone or granuloma in the human gastro-intestinal tract. *Acta medica et biologica* **22**, 57–98.

Shiraki, T., Suzuki, T., Otsuru, M., Sato, Y., Kenmotsu, M. and Asaishi, K. (1973). (Experiments for the application of fluorescent antibody method to histological diagnosis of anisakiasis. 1. Reactivity of anti-*Anisakis* crude antigen IgG and anti-*Anisakis* purified antigen IgG to sections of *Anisakis* larvae under various conditions.) *Japanese Journal of Parasitology* **22**, 131–140. (In Japanese.)

Shiraki, T., Hasegawa, H. and Kenmotsu, M. (1976). Larval anisakid nematodes from the prawns, *Pandalus* spp. *Japanese Journal of Parasitology* **25**, 148–152.

Sindermann, C. J. (1961a). Parasite tags for marine fish. *Journal of Wildlife Management* **25**, 41–47.

Sindermann, C. J. (1961b). Parasitological tags for redfish of the western North Atlantic. *Rapport et procès-verbaux des réunions. Conseil permanent international pour l'exploration de la mer* **150**, 111–117.

Skryabin, A. S. (1959). (New species of helminths from marine mammals from the Pacific Ocean and Far-eastern Seas.) *Izvestiya Crimskogo Pedagogicheskogo instituta* **34**, 99–118. (In Russian.)

Sluiters, J. F. (1974). *Anisakis* sp. larvae in the stomachs of herring (*Clupea harengus* L.). *Zeitschrift für Parasitenkunde* **44**, 279–288.

Smith, J. W. (1971). *Thysanoessa inermis* and *T. longicaudata* (Euphausiidae) as first intermediate hosts of *Anisakis* sp. (Nematoda: Ascaridata) in the northern North Sea, to the north of Scotland and at Faroe. *Nature, London* **234**, 478.

Smith, J. W. (1974). Experimental transfer of *Anisakis* sp. larvae (Nematoda: Ascaridida) from one fish host to another. *Journal of Helminthology* **48**, 229–234.

Smith, J. W. and Wootten, R. (1975). Experimental studies on the migration of *Anisakis* sp. larvae (Nematoda: Ascaridida) into the flesh of herring, *Clupea harengus* L. *International Journal for Parasitology* **5**, 133–136.

Sommerville, R. I. (1974). Physico-chemical requirements for development *in vitro*

of *Anisakis* sp. larvae from fish. (Abstract.) *Proceedings of the 3rd International Congress of Parasitology, Munich, Aug. 25–31, 1974* **1**, 461–462.

Sommerville, R. I. and Buzzell, G. R. (1974). Nematode parasites of fish. *Tasmanian Fisheries Research* **8**, 26–27.

Sommerville, R. I. and Davey, K. G. (1976). Stimuli for cuticle formation and ecdysis *in vitro* of the infective larva of *Anisakis* sp. (Nematoda: Ascaridoidea). *International Journal for Parasitology* **6**, 433–439.

Soulsby, E. J. L. (1976). Serodiagnosis of other helminth infections. *In* "Immunology of Parasitic Infections" (S. Cohen and E. Sadun, eds), pp. 152–161. Blackwell Scientific Publications, Oxford, London, Edinburgh and Melbourne.

Sprent, J. F. A. (1969). Helminth "zoonoses": an analysis. *Helminthological Abstracts* **38**, 333–351.

Stern, J. A., Chakravarti, D., Uzmann, J. R. and Hesselholt, M. N. (1958). Rapid counting of Nematoda in salmon by peptic digestion. *U.S. Fish and Wildlife Service, Special Scientific Report—Fisheries,* No. **255**, 5 pp.

Suzuki, T. (1968). (Studies on the immunological diagnosis of anisakiasis: I. Antigenic analysis of *Anisakis* larvae by means of electrophoresis.) *Japanese Journal of Parasitology* **17**, 213–220. (In Japanese.)

Suzuki, T., Shiraki, T. and Otsuru, M. (1969). (Studies on the immunological diagnosis of anisakiasis. II. Isolation and purification of *Anisakis* antigen.) *Japanese Journal of Parasitology* **18**, 232–240. (In Japanese.)

Suzuki, T., Shiraki, T., Sekino, S., Otsuru, M. and Ishikura, H. (1970). (Studies on the immunological diagnosis of anisakiasis. III. Intradermal test with purified antigen.) *Japanese Journal of Parasitology* **19**, 1–9. (In Japanese.)

Suzuki, T., Shiraki, T. and Otsuru, M. (1971). Immunodiagnosis of anisakiasis, with special reference to purification of specific antigen. *Chinese Journal of Microbiology* **4**, 217–231.

Suzuki, T., Sato, Y., Yamashita, Y., Sekikawa, H. and Otsuru, M. (1974). Anisakiasis: preparation of a stable antigen for indirect fluorescent antibody test. *Experimental Parasitology* **35**, 418–424.

Sweeney, J. C. and Ridgway, S. H. (1975). Common diseases of small cetaceans. *Journal of the American Veterinary Medical Association* **167**, 533–540.

Tanaka, H., Takata, S., Nishimura, T. and Watanabe, S. (1968). (A case report of *Anisakis* larva penetration in the pharynx mucosa.) *Japanese Journal of Parasitology* **17**, 641. (In Japanese.)

Taniguchi, M. (1966). (Studies on anisakiasis. I. On its antigenicity.) *Japanese Journal of Parasitology* **15**, 502–506. (In Japanese.)

Taniguchi, M. (1967). (Studies on *Anisakis*. II. On amylase-isozyme.) *Japanese Journal of Parasitology* **16**, 114–118. (In Japanese.)

Taniguchi, M. (1970a). Homocytotropic antibody of rabbits, sensitized or infected with *Anisakis* (Nematoda).) *Japanese Journal of Parasitology* **19**, 189–195. (In Japanese.)

Taniguchi, M. (1970b). (Comparative study on *Anisakis* adult and larva antigens.) *Japanese Journal of Parasitology* **19**, 254–257. (In Japanese.)

Templeman, W., Squires, H. J. and Fleming, A. M. (1957). Nematodes in the fillets of cod and other fishes in Newfoundland and neighbouring areas. *Journal of the Fisheries Research Board of Canada,* **14**, 831–897.

Terry, R. J. (1968). Applications of immunology to helminth diseases. *In* "Immunity to Parasites" (A. E. R. Taylor, ed.), pp. 91–103. 6th Symposium of the British Society for Parasitology. Blackwell Scientific Publications, Oxford, London, Edinburgh and Melbourne.

Testi, F., Bravi, M. V. and Andreucci, A. (1971). Gastrite ulcerativa da nematodi

in un delfino (*Delphinus delphis* L.) del mare Adriatico. *Parassitologia* **13**, 355–360.

Toussaint, A. J. and Anderson, R. I. (1965). A soluble antigen fluorescent antibody (SAFA) technique. *Applied Microbiology* **13**, 552-558.

Tsuji, M. (1975). (Comparative studies on the antigenic structure of several helminths by immunoelectrophoresis.) *Japanese Journal of Parasitology* **24**, 227–236. (In Japanese.)

Uspenskaya, A. V. (1955). (The parasite fauna of benthic crustaceans of the Barents Sea.) *Report. Leningrad State University*, 3–16. (In Russian.)

Uspenskaya, A. V. (1960). Parasitofaune des crustacês benthiques de la mer de Barents. (Exposé préliminaire). *Annales de parasitologie humaine et comparée* **35**, 221–242.

Uspenskaya, A. V. (1963). (Parasite fauna of benthic crustaceans from the Barents Sea.) Izdatel'stvo Akademiya Nauk SSSR, Moscow and Leningrad, 127 pp. (In Russian.)

Usui, M., Ashizawa, H., Nosaka, D. and Tateyama, S. (1973). (Studies on swine gastric anisakiasis. 1. Morphological findings on worms.) *Bulletin of the Faculty of Agriculture, Miyazaki University* **20**, 169–177. (In Japanese.)

Van Banning, P. (1971). Some notes on a successful rearing of the herring-worm, *Anisakis marina* L. (Nematoda: Heterocheilidae). *Journal du Conseil* **34**, 84–88.

Van Mameren, J. and Houwing, H. (1967). Effect of irradiation on *Anisakis* larvae in salted herring. *In* "Elimination of harmful organisms from food and feed by irradiation." Proceedings of a panel organized by the joint FAO/IAEA division of atomic energy in food and agriculture, Zeist, 12–16 June, 1967, pp. 73–80.

Van Thiel, P. H. (1962). Anisakiasis. (Abstract.) *Parasitology* **52**, (Suppl.), 16P–17P.

Van Thiel, P. H. (1966). The final hosts of the herring-worm *Anisakis marina*. *Tropical and Geographical Medicine* **18**, 310–328.

Van Thiel, P. H. (1967). Le phénomène de la "formation de la calotte" chez la larve d'*Anisakis marina* (Linnaeus 1767) dans du sérum humain. *Annales de parasitologie humaine et comparée* **42**, 633–642.

Van Thiel, P. H. (1976). The present state of anisakiasis and its causative worms. *Tropical and Geographical Medicine* **28**, 75–85.

Van Thiel, P. H. and Van Houten, H. (1967). The localization of the herring-worm *Anisakis marina* in—and outside the human gastro-intestinal wall (with a description of the characteristics of its larval and juvenile stages). *Tropical and Geographical Medicine* **19**, 56–62.

Van Thiel, P. H., Kuipers, F. C. and Roskam, T. H. (1960). A nematode parasitic to herring, causing acute abdominal syndromes in man. *Tropical and Geographical Medicine* **12**, 97–113.

Viglierchio, D. R. and Görtz, J. H. (1972a). *Anisakis physeteris:* amino acids in body tissues. *Experimental Parasitology* **32**, 140–148.

Viglierchio, D. R. and Görtz, J. H. (1972b). Hemoglobin derivatives of the zooparasitic nematode *Anisakis physeteris* and the sperm whale host. *Experimental Parasitology* **32**, 211–216.

Vik, R. (1964). Penetration of stomach wall by *Anisakis*-type larvae in porpoises. *Canadian Journal of Zoology* **42**, 513.

Vik, R. (1966). *Anisakis* larvae in Norwegian food fishes. (Abstract) *Proceedings of the 1st International Congress of Parasitology, Sept. 21–26, 1964*, **1**, 568–569

Vivares, C. P. (1972/73). Le parasitisme chez les brachyoures (Crustacea, Decap-

oda) de la Côte Méditerranéenne Française et des étangs du Languedoc-Roussillon. *Vie et Milieu, séries A* **23**, 191–218.

Vivares, C. P. (1973). Étude du parasitisme des crustacés décapodes brachyoures en Méditerranée occidentale: premiers résultats. *Rapport et procès-verbaux des réunions. Commission internationale pour l'exploration scientifique de la Mer Méditerranée* **21**, 661–663.

Warren, K. S. (1974). Modulation of immunopathology in schistosomiasis. *In* "Parasites in the Immunized Host: Mechanisms of Survival." CIBA Foundation Symposium 25 (new series). Associated Scientific Publishers, Amsterdam, 280 pp.

Wheeler, A. (1969). "The Fishes of the British Isles and North-west Europe." MacMillan, London, Melbourne and Toronto.

Williams, H. H. and Jones, A. (1976). Marine helminths and human health. CIH Miscellaneous publication No. 3, 47 pp. Commonwealth Agricultural Bureaux, Farnham Royal, Bucks, England.

Williams, H. H. and Richards, D. H. H. (1968). Observations on *Pseudanisakis rotundata* (Rudolphi, 1819) Mozgovoi, 1950, a common but little known nematode parasite of *Raia radiata* Donovan in the Northern North Sea. *Journal of Helminthology* **42**, 199–219.

Wootten, R. and Smith, J. W. (1975). Observational and experimental studies on the acquisition of *Anisakis* sp. larvae (Nematoda: Ascaridida) by trout in fresh water. *International Journal for Parasitology* **5**, 373–378.

Wootten, R. and Smith, J. W. (1976). Observational and experimental studies on larval nematodes in blue whiting from waters to the west of Scotland. *ICES Pelagic Fish (Northern) Committee*, C. M. 1976/H: 35, 7 pp.

Wootten, R. and Waddell, I. F. (1977). Studies on the biology of larval nematodes from the musculature of cod and whiting in Scottish waters. *Journal du Conseil* **37**, 266–273.

Wright, D. J. and Newall, D. R. (1976). Nitrogen excretion, osmotic and ionic regulation in nematodes. *In* "The Organization of Nematodes" (N. A. Croll, ed.), pp. 163–210. Academic Press, New York and London.

Wu, C. S. (1970). Histological studies on monkeys experimentally infected with *Anisakis* larvae. *Chinese Journal of Microbiology* **3**, 29–41.

Yamaguchi, T., Chen, E. R., Hsieh, H. C. and Shih, C. C. (1970). Experimental infection of *Anisakis* larvae in Taiwan monkeys with results of examinations of marine fishes of Taiwan for the parasite. *Journal of the Formosan Medical Association* **69**, 371–377.

Yasuraoka, K., Koyama, T. and Kato, K. (1967). (Studies on the *in vitro* axenic development of *Anisakis* larvae. (I).) *Japanese Journal of Parasitology* **16**, 290–291. (In Japanese.)

Yokogawa, M. and Yoshimura, H. (1967). Clinicopathologic studies on larval anisakiasis in Japan. *American Journal of Tropical Medicine and Hygiene* **16**, 723–728.

Young, P. C. (1972). The relationship between the presence of larval anisakine nematodes in cod and marine mammals in British home waters. *Journal of Applied Ecology* **9**, 459–485.

Young, P. C. and Lowe, D. (1969). Larval nematodes from fish of the subfamily Anisakinae and gastro-intestinal lesions in mammals. *Journal of Comparative Pathology* **79**, 301–313.

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Prospects for the Development of Dead Vaccines against Helminths

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I. Introduction

Vaccination against smallpox by inoculation of the contagious material from pustules of cowpox, or vaccinia (vacca = cow) was introduced by Jenner in 1798 and by 1858 its use was compulsory in Britain. In the 1880s Pasteur's discovery of simple methods for attenuating bacteria, including culturing under abnormal conditions, opened the way for development of a live vaccine against Anthrax. In the same period, Wright and Pasteur himself, pioneered the use of dead bacteria in vaccines against typhoid, cholera and plaque. These two methods have formed the basis for nearly all subsequent vaccines against bacterial or viral diseases. The exceptions are the diphtheria and tetanus vaccines developed in the early years of this century, which involved immunization with an inactivated form of the exotoxins released by the bacteria during culture.

It is not immediately obvious why these basically simple methods which led to such spectacular advances in the control of infectious microbial diseases were not more quickly tested against diseases caused by metazoan helminth parasites, the nematodes, cestodes and trematodes. One reason is that parasitic helminths rarely cause acute disease with high mortality in either man or livestock. A disease caused by the presence of helminths is likely to produce a chronic debilitating condition in which symptoms develop gradually. This factor has undoubtedly contributed to the slow development of an awareness of the importance of helminth diseases to human and animal health, particularly in the developing tropical countries in which most of the more serious helminthiases occur. A second reason was the slow recognition that more than marginal levels of acquired immunity are developed against many parasitic helminths.

An important feature of most helminth diseases is the long persistence of some adult parasites in hosts which have acquired high levels of resistance to new infections. Evasion of the immune effector mechanisms by some of the parasites derived from a primary infection tends to obscure the development of resistance to reinfection.

The first attempts to vaccinate against parasitic helminths with dead whole organisms or extracts were made in the 1930s and although a few results seem to have been promising enough to warrant further investigation, most were rather disappointing and interest waned until the 1950s. At this time Thorson (1953) carried out successful vaccination against a nematode using antigenic material secreted by living worms. The first vaccine based on attenuated organisms was developed soon afterwards. Larvae of the cattle lungworm were X-irradiated at a level which prevented full development but allowed induction of a high degree of resistance to a challenge with normal larvae (Jarrett *et al.*, 1960).

Reviews of the field soon after these developments (Stoll, 1961; Thorson, 1963; Soulsby, 1963) predicted rapid development of both live vaccines based on attenuation by irradiation and dead vaccines using antigens secreted into culture media. Unfortunately, these advances have not materialized. The irradiated lungworm vaccine has been in commercial production for 17 years and the method has been tested with some success with a range of other helminths but so far only one other vaccine, against the dog hookworm, has reached the market (Miller, 1971; 1978, this volume). Mulligan (1975) suggests several reasons why irradiated vaccines have not come into greater use. The most important of these is the limited shelf-life of most irradiated helminth larval forms and the fact that a live vaccine is likely to be applicable only in those diseases in which a high level of immunity can be induced by a natural infection. Although there has been considerable progress with experimental dead vaccines based on the use of antigens secreted into culture medium (reviewed by Thorson, 1970; Silverman, 1970) no commercial vaccine has yet been developed.

Even a brief survey of the literature shows quite clearly that very much more research effort has been directed towards solving the mechanisms of immunity against parasitic helminths than to direct empirical attempts to

develop vaccines. The concentration of research on mechanisms of immunity is understandable. Unravelling of the complex immune responses which are capable of destroying large multicellular parasites is intrinsically challenging and has, in fact, yielded some information of general interest in immunology. Examples are the role of the eosinophil in anti-schistosome reactions (Mahmoud *et al.*, 1975) and the mechanisms evolved by many parasites for evasion of the immune response (Ciba Symposium, 1974). As far as the objective of immunoprophylaxis is concerned, analysis of the mechanisms implicated in helminth immunity can be expected to indicate the kind of reactions to which the parasite is susceptible and eventually to definition of the target antigens. When identified, these antigens can be purified in large quantities and used to stimulate, artificially, a state of immunity similar to that induced by a natural infection. So far, however, this approach has not produced any information leading to successful experimental vaccination against a parasite.

The present review is restricted to a consideration of the empirical approach to the development of dead vaccines which has had a chequered history but shows some current promise of success. Consideration is also given to some of the many problems yet to be solved before experimental vaccination can be translated from the laboratory to actual production.

II. Nematodes

A. Introduction

Most species of nematode are free-living organisms found in a very wide range of habitats. The parasitic forms are also numerous and occur in most animals and plants. Parasitic nematodes do not multiply within the host. In vertebrates most nematodes are found in the gastrointestinal tract and the large numbers of eggs produced by the adult worms nearly always develop externally into free-living stages which may then infect new hosts. Some parasitic nematodes, like the filarial worms, are tissue parasites transmitted by an insect vector.

The extent of human and animal ill-health caused by parasitic nematodes is not easy to assess. Most diseases caused by nematodes are of a chronic debilitating nature and result in relatively few deaths. Consequently crude mortality and morbidity statistics offer a very poor guide to the significance of diseases caused by parasitic nematodes and this has been freely acknowledged in some of the attempts to compile such data (Tropical Health, 1962). In addition most nematode diseases of man occur in tropical or sub-tropical developing countries which often do not have the facilities for mass diagnosis of parasitic diseases. Stoll (1947) pointed out many of these restrictions in his classic paper on the general level of helminthiases in the world and his estimates are probably still the best available and most frequently quoted (Cavier and Hawking, 1973). In a total population which was then about 2000 million, Stoll assessed the number of human cases of parasitism by nematodes at around 1700 million. Some of these parasites do not cause ill-health but hookworm disease was estimated to affect 456 million people, Ascariasis some

644 million and filarial infections around 209 millions. With the enormous expansion of world population in the last 30 years these estimates are now likely to be conservative even though there have been considerable advances in the development of effective drugs. The loss of production in domestic livestock due to disease caused by nematodes is generally considered to be of a high order even in the advanced countries where drugs are commonly used in prophylactic regimes and it is certainly more serious in the under-developed regions (Lindahl *et al.*, 1963; Jarrett and Urquhart, 1971).

The immune response of the host to parasitic nematodes may act on the larval or adult stages of the worm in a variety of ways. Adult nematodes may be expelled from the intestine or destroyed in the tissues but in some cases the immune reaction may cause stunting in size or interference with egg-laying capacity. Larvae of a second or subsequent infection may be killed during migration or temporarily arrested in development. The mechanisms of the immune response have been most thoroughly investigated in model systems using laboratory hosts, particularly *Nippostrongylus brasiliensis* in the rat (reviewed by Ogilive and Love, 1974; Ogilvie and Worms, 1976). In this system expulsion of the adult worm from the intestine involves several immune effector mechanisms. Antibody (IgG_1) damages adult worms in some way causing visible deterioration in the cytoplasm of cells in the gut and reproductive system but these effects are probably secondary to inhibition of enzyme secretion by the parasite. Following damage by antibody, expulsion of the worms is dependent on a second step involving specifically sensitized lymphocytes and a cell or cells derived from the bone marrow. The bone marrow component may be a basophil, macrophage or eosinophil since all these cell types, as well as mast cells, are present in increased numbers in the lamina propria close to the parasites at the onset of the second step in the expulsion process. No essential role for IgE (reaginic antibody) mast cells or eosinophils has yet been defined in this system but the general association of these elements with nematode infections suggests an important role for IgE-mediated anaphylaxis in the rejection mechanism (reviewed by Jarrett and Urquhart, 1971; Murray, 1972).

Progress with vaccination against nematodes has been based largely on direct empirical attempts to protect laboratory animals with antigen preparations derived from whole parasites or their secretions (reviewed by Soulsby, 1966; Thorson, 1970; Silverman, 1970; Ogilvie and Worms, 1976). In the earliest work on vaccination, a rather arbitrary classification of antigens was introduced and many of the terms have persisted in the literature. Homogenates or extracts of whole nematodes were considered to contain "somatic antigens". Materials released into culture medium were described as "excretory/secretory" (ES) antigens or sometimes "metabolic" or "culture fluid" antigens. For simplicity the term secreted (or secretory) antigens has been used in this review. Since they originate in glandular tissue within the parasite, "secreted" antigens are usually present in "somatic" antigen preparations and this term has not been used. The crucial antigen responsible for inducing a protective immune response in the host has been described most frequently as "functional antigen" and we have used this term throughout.

B. VACCINATION WITH WHOLE WORM MATERIAL: EARLY WORK

Attempts to vaccinate against parasitic nematodes with dead whole organisms, homogenates or extracts began in the 1930s. Sheldon (1937) successfully vaccinated rats against *Strongyloides ratti* by giving a total of 16 000 heat-killed larvae in thirteen subcutaneous injections at intervals of three days. The resulting resistance to a challenge infection was "almost as great as that resulting from a single infection with 1000 living larvae". In spite of this early success, most other attempts to vaccinate against nematodes with dead organisms or extracts from them were either ineffective or only very moderately successful. Kerr (1938) could not detect any increased resistance to the dog hookworm *Ancylostoma caninum* in mice or *Ascaris suum* in guinea-pigs following repeated vaccination with heat-killed larvae or extracts prepared in various ways. Similarly vaccination of dogs with powdered antigen prepared from dried *Dirofilaria immitis* was completely unsuccessful (Feng, 1937; Murata, 1939). McCoy (1935) was able to show a slight protective effect against *Trichinella spiralis* in rats vaccinated with heat-killed or dried powdered larvae but Bachman and Molina (1933) could not demonstrate any protection against the same parasite in similarly vaccinated pigs. Chandler (1932) and Watt (1943) both detected some degree of protection in rats vaccinated with heat-killed larvae of *Nippostrongylus muris* but the effect demonstrated was restricted to a marked reduction in egg production and the number of adult worms recovered from vaccinated rats after challenge did not differ significantly from control values. Sprent and Chen (1949) did not detect any resistance against *Ascaris lumbricoides* in mice following vaccination with homogenates of worm tissues.

The failure, or at most, marginal success, of the majority of these early attempts to vaccinate against parasitic nematodes gave rise to the view that the antigens needed to stimulate an effective immune response, the functional antigens, were not present in sufficient quantity in homogenates of dead worms, but might be actively secreted by the living parasite.

C. VACCINATION WITH SECRETED ANTIGENS

1. *Strongylid nematodes*

The idea that the functional antigens of parasitic nematodes might be present in secretions derived considerable support from the observation that when *Nippostrongylus brasiliensis* was maintained in serum from rats immunized by repeated infections, precipitates were formed at the mouth, excretory pore and anus of the worm (Sarles, 1938; Taliaferro and Sarles, 1939). Similar precipitates were detected histologically around the orifices of larval worms in the skin and lungs of immune rats. These observations indicated that precipitating antibodies directed against secretions (and possibly excretions) of the parasite were present in immune serum. It was suggested that although the antigen–antibody complexes formed at the orifices of the parasite did not appear to cause direct damage, they might be responsible for blocking

the activity of some secretion which was essential for the maintenance of the parasite, possibly an enzyme involved in feeding. This idea was originally suggested by Chandler (1936). A number of other workers demonstrated similar precipitates around the orifices of a variety of nematodes when they had been maintained in immune serum from animals given several previous homologous infections (see Thorson, 1970). However, few attempts to vaccinate animals with secretions collected from nematodes seem to have been made until Thorson (1953) showed that rats could be partially protected against a challenge with *Nippostrongylus* by vaccination with secretory products released into serum or saline by larval parasites during a short period of maintenance *in vitro*. In these experiments as many as 4×10^6 living larvae were incubated in balanced saline at room temperature for 48 h or in rat serum at 37° for 24 h. Rats were vaccinated by six intraperitoneal injections of 0·1–0·3 ml of the medium containing secretions on alternate days and a challenge was given four days later. Secretions obtained in both ways gave approximately 60% protection against a challenge which is considerably below the level obtainable by repeated experimental infection with living larvae. Very recently (Poulain *et al.*, 1976) the effectiveness of secretions collected *in vitro* for vaccination of rats against *Nippostrongylus brasiliensis* has been confirmed. These workers harvested large quantities of antigen secreted by larvae of *Nippostrongylus* maintained in balanced saline for 48 h and vaccinated rats by introducing 2 mg of lyophilized secretions directly into the stomach. High levels of resistance equivalent to 92% protection against challenge were induced in this way but extracts of soluble antigens from larvae were relatively ineffective. No evidence was presented to indicate whether the high level of protection obtained with secretions was due to the route of administration which is similar to the natural route in an infection. It seems quite possible that antigen entering the host through the gut is more efficient in stimulating immunity and some evidence pointing in this direction has been obtained by Hepler *et al.* (1976) who showed that ten times as many living larvae of *Nematospiroides dubius* are needed to immunize mice when given subcutaneously rather than by the oral route.

2. *Nature and origin of strongylid secreted antigens*

Thorson (1953) showed that the formation of precipitates at the orifices of living *Nippostrongylus* larvae in immune rat serum could be partially prevented by previous absorption of the serum with secretions collected *in vitro*. Similarly, serum from rats vaccinated with secretions caused the formation of the characteristic precipitates indicating the close similarity of antigens secreted *in vitro* to those released by the parasite in the host. Lipolytic enzyme activity was detected in secretions collected *in vitro* and the enzyme was inhibited by antiserum from rats hyperimmune to *Nippostrongylus* (Thorson, 1954).

In an attempt to determine the origin of the secreted antigens Thorson (1956a; 1963) used the dog hookworm *Ancylostoma caninum* because it was found to be relatively easy to dissect out the oesophagus with its associated

glands from this species. Extracts of oesophageal tissue from *Ancylostoma* contained proteolytic and lipolytic enzymes and a peptidase. Serum from dogs given repeated natural infections partly blocked these enzymic activities. Although vaccination of dogs with extracts of oesophageal tissue gave moderate levels of protection against challenge with *Ancylostoma* (Thorson 1956b) this demonstration that the functional antigens are probably enzymes secreted by glands associated with the oesophagus had considerable impact on later work with nematodes. The significance for the parasite of the enzymes secreted by the oesophageal glands is unknown but they may well be important in nutrition (Chandler, 1936; Thorson, 1956a).

Very little further work on enzymes secreted by nematodes seems to have been carried out until Sanderson and Ogilvie (1971) showed that *Nippostrongylus brasiliensis* released substantial quantities of acetylcholinesterase (AchE) when maintained *in vitro*. This enzyme is usually associated with nerve transmission but Lee (1970) using histochemical techniques found concentrations in the excretory glands and oesophageal glands of *Nippostrongylus* as well as in the nerve ganglia. The so-called excretory glands of nematodes are large paired ventral glands which open to the exterior through an anterior ventral excretory pore.

Ogilvie *et al.* (1973) subsequently demonstrated the presence of AchE in a wide range of gastrointestinal nematodes. The existence of antibodies directed against parasite AchE in the sera of infected hosts suggests but does not prove that the parasites actually secrete the enzyme *in vivo*. Intriguing evidence that secretion does occur in the host has been obtained for *Nippostrongylus brasiliensis* in which the AchE is secreted in several isoenzymic forms. As immunity develops during an infection and antibodies are formed against the predominant AchE isoenzyme, the parasite begins to secrete an alternative isoenzyme in greater quantities (Edwards *et al.*, 1971; Jones and Ogilvie, 1972).

The possible function of acetylcholinesterase secreted by gastrointestinal nematodes remains a matter for speculation. It has been suggested that this enzyme may act as a biochemical "holdfast" by inhibiting peristalsis in a localized region of the intestine and thereby preventing expulsion of the parasites from this site (Ogilvie and Jones, 1971).

Chromatographic analysis of acetylcholinesterase extracted from *Nippostrongylus brasiliensis* and *Trichostrongylus colubriformis* has indicated a molecular weight in the region of 70000 daltons which allows the enzyme to be separated on a size basis from allergens which are restricted to a range between 10000 and 50000 (Hogarth-Scott *et al.*, 1973). Rothwell and Merritt (1975) have tested soluble fractions of *T. colubriformis* rich in either AchE or allergen for vaccination of guinea-pigs. A fraction with much AchE did not protect at all, but a second fraction with less than 0·001 times this amount of AchE gave a high level of protection against challenge. This evidence clearly suggests that parasite acetylcholinesterase is not a functional antigen. The fraction which gave effective vaccination contained allergens but these could not be positively identified as functional antigens without further purification. The possibility that allergens of *Trichostrongylus* may prove to be

functional antigens is very intriguing since these are the antigens involved in stimulating the production of IgE which mediates anaphylactic-type hypersensitivity reactions. The possible involvement of this type of response in the immune rejection of parasitic nematodes has been repeatedly suggested (Jarrett and Urquhart, 1971; Murray, 1972).

3. *Secretory antigens in trichuroid nematodes*

Trichinella spiralis has been used frequently for experimental work on vaccination, partly because it is one of the few nematodes affecting man which can be readily maintained in laboratory hosts and partly because a single infection stimulates a very high level of immunity to reinfection (Larsh, 1961). *T. spiralis*, an intestinal parasite of pigs and some other carnivores, is unusual in that its eggs hatch within the body of the female and the larvae migrate through the gut wall and disperse into the skeletal musculature where they encyst. The life cycle is completed when pigs eat infected pig meat and man can be accidentally infected from the same source without contributing to maintenance of the cycle.

Antigenic material secreted by excysted muscle larvae of *T. spiralis* was used by Spindler (1937) to vaccinate rats, rabbits and guinea-pigs by oral administration of a clarified digest of infected muscle tissues. Although only a minor degree of protection was achieved, this probably represents the earliest attempt to vaccinate against a nematode with secreted antigens. Following the more successful vaccination results with secreted antigens of *Nippostrongylus* Campbell (1955) attempted vaccination of mice with antigens secreted into culture medium by larval *Trichinella*. The medium contained serum ultrafiltrate to avoid contamination of secreted antigens with serum proteins and larvae derived from muscle digests were maintained for five days at a concentration of 2500 ml^{-1}. Mice were given a single subcutaneous dose of this five day old culture fluid.

Following a challenge infection with living larvae, there was no significant reduction in the number of adult worms which developed in vaccinated mice compared with controls but the number of encysted muscle larvae, derived from these adults, was reduced by approximately 50%. In addition, the adult worms were expelled more rapidly in previously-vaccinated mice than those in a normal primary infection and some stunting of female worms was observed. Essentially similar results were obtained with secreted antigens derived from larvae by Chute (1956) and Ewart and Olson (1961). Mills and Kent (1965) employed a novel technique to examine the role of secreted antigens. Larvae of *T. spiralis* were incubated in anti-*Trichinella* serum from immune rabbits before being used to infect normal rabbits. Significantly fewer of the larvae treated with specific antibodies developed in comparison with larvae treated with normal rabbit serum or immune serum previously absorbed with antigens secreted into culture medium by larval *T. spiralis*.

Chipman (1957) also demonstrated that antigens secreted by adult *T. spiralis* could be effective in vaccinating mice. Six daily injections of culture fluid in which adult worms had been maintained for two days were given by

intraperitoneal injection and a marked reduction in the numbers of encysted muscle larvae was observed following a challenge infection. A longer course of injections with secreted antigens induced an immune response which also affected the number and size of the adult worms developing from a challenge.

Early attempts to vaccinate with dead whole larvae or extracts yielded very variable results. McCoy (1935), Shikhobalova (1953) and Kozar (1969) reported a limited degree of vaccination but Bachman and Molina (1933) could not protect pigs with dead material. Berntzen (1974) found that soluble antigens extracted from larvae or adults did not reduce the level of larvae in the muscles of rats following a challenge but secreted antigens harvested from medium used to culture larvae to the adult stage were highly effective.

Direct evidence for the involvement of secreted antigens *in vivo* was obtained by the demonstration that a high level of immunity could be induced in mice by larvae contained in millipore diffusion chambers implanted in the peritoneal cavity for seven days (Despommier and Wostmann, 1968). These results, taken together, indicated strongly that the functional antigens of *T. spiralis* are secreted into culture medium by living larval and adult stages of the parasite.

4. *Isolation of trichuroid functional antigens*

The failure of material derived from dead larvae or adults to vaccinate was interpreted as implying that the secretory antigens were synthesized by cultured parasites but not stored in any appreciable quantity in glandular tissue. More recent work by Despommier and colleagues has shown that this interpretation is incorrect; secretory granules can be isolated from dead larval *T. spiralis* which give very high levels of protection in mice (Despommier and Müller, 1970b). The secretory granules are derived from an unusual organ present in all trichuroid nematodes, the stichosome. This consists of a single column of cells lying dorsal to a very narrow oesophagus into which they open by very fine ducts (Despommier and Müller, 1976). The cells, or stichocytes, are of two types containing either α or β secretion granules, which can be partially purified from cell-free homogenates of larval worms by differential centrifugation followed by isopycnic centrifugation in a continuous sucrose density gradient (Despommier and Müller, 1970a; 1976). The two types of secretion granules, distinguished morphologically by electron microscopy, each contain four unique antigens detected by Ouchterlony immunodiffusion against anti-*Trichinella* serum from hyperimmune rabbits. These same antigens were all detectable in material secreted into culture medium by living muscle larvae and hyperimmune serum labelled with horse-radish peroxidase was employed to show that the secretion granules were localized in the cytoplasm of the stichocytes (Despommier and Müller, 1976). So far, no information on the chemical nature of the granules is available and their function remains a matter for speculation. The granules appear to be secreted into the oesophagus during larval migration and during limited penetration of the intestinal mucosa by adults. On this basis an enzymic role assisting migration

through the tissues has been suggested (Bruce, 1974) but the secretion may well contain enzymes involved in nutrition as suggested for oesophageal secretions in other nematodes.

In a preliminary report Despommier and Müller (1970b) showed that as little as 10 μg of a fraction containing mainly β granules given once with Freund's complete adjuvant could depress the level of encysted larvae in the musculature of mice, following a challenge, by 95%. A reduction of 98% could be obtained if the dose of antigen was increased to 100 μg.

Recently, using fractions prepared on a larger scale (Despommier *et al.*, 1974) which contained both types of granule, these results in mice have been essentially confirmed (Despommier *et al.*, 1977). With 100 μg of antigen given intraperitoneally with Freund's complete adjuvant in three equal doses at weekly intervals, the numbers of muscle larvae resulting from a challenge were depressed by 78% in mice but only by 40% in rats receiving a total of 250 μg of the antigenic material. Vaccination in this way with secretory granules derived from the stichosome apparently mainly affects the fecundity of female *T. spiralis*. Worms recovered from vaccinated mice or rats produced far fewer larvae *in vitro* than worms of comparable age from normal hosts and this depression in production of larvae correlates well with the reductions observed in the numbers of larvae encysted in the musculature (Despommier *et al.*, 1977). When higher doses of antigen were used in mice (100 μg) reductions in the numbers of adult females were also observed.

Other trichuroid nematodes have a stichosome similar to that of *T. spiralis* which occupies most of the anterior region of the body. Mechanical separation of the whole of this anterior stichosome region from the rest of the body forms a simple initial method of fractionation and soluble antigens extracted from the stichosome of *Trichuris muris* have been used to vaccinate mice very effectively (Wakelin and Selby, 1973). As little as 100 μg of total soluble protein given in a single intramuscular dose with Freund's incomplete adjuvant resulted in a 92% reduction in the numbers of adult worms developing from a challenge infection. Progress towards the isolation of the soluble functional antigen has now been made (Jenkins and Wakelin, 1977). The stichocytes of *T. muris* were squeezed out of the anterior end of the worm and an extract of the cells contained soluble protein which induced a high level of protection in mice. Soluble material secreted by worms incubated for 3 h at 37° C in saline also vaccinated efficiently. Immunodiffusion and immunoelectrophoresis gave a single precipitin line in the stichocyte extract which showed identity with a single line in secreted material. The same antigen could be detected among several other components in whole worm extract. Treatment of whole worm extract with protelytic enzymes destroyed immunogenicity but sodium metaperiodate had no effect suggesting that the functional antigen is a protein without a significant carbohydrate moiety. One of the functional antigens of *T. muris* is evidently a soluble protein derived from the stichocytes and further studies may be expected to show whether this is the only functional antigen and whether it originates in the granules known to be present in the stichocytes.

5. *Secretory antigens associated with moulting*

In one of the first investigations of the immune response to gastrointestinal nematodes in cattle and sheep, observations were made which strongly suggested that an immune reaction capable of blocking egg production and causing expulsion of many adult worms was triggered by an early stage in the development of a challenge infection (Stoll 1929). This so-called "self cure" reaction has been most studied in the response of sheep to the stomach-worm *Haemonchus contortus*. After sensitization of sheep by several infections, a further challenge can result in a local hypersensitivity reaction in the abomasum which causes rejection of most of the adult worms from this site (Stewart, 1953). The reaction occurs at a time when the larvae of the challenge exposure are moulting from the third to the fourth larval stage between two and four days after infection (Soulsby and Stewart, 1960). Similarly adult worms in infections of pigs or laboratory hosts with *Ascaris suum* may be expelled by a self-cure mechanism (Taffs, 1968) and the larvae of a challenge infection in an immune host are affected only when they approach the moulting period (Soulsby, 1961).

The apparent involvement of antigens secreted during the moulting process with the initiation of immune reactions directed against adult and larval stages led to attempts to vaccinate with antigenic material secreted by larval nematodes which had undergone moulting during culture *in vitro*. Silverman *et al.* (1962) cultured the gastrointestinal nematode, *Trichostrongylus colubriformis* and the lungworm *Dictyocaulus viviparus* in a simple medium (Silverman, 1959) consisting of Ringer's saline supplemented with a liver extract. Second-stage infective larvae derived from faecal cultures from infected sheep were cultured through two moults until 60% of the larvae had reached the fourth stage. The culture medium and larvae were lyophilized for storage. Maximum levels of protection against challenge in guinea-pigs (96%) required two intraperitoneal injections of antigen derived from 2500 larvae at an interval of 21 days. Lower levels of antigen obtained from 1000 larvae were less effective but administration with alhydrogel adjuvant gave more than 90% protection. Lyophilized antigen stored at room temperature for up to six months showed no loss of activity. High levels of protection in guinea-pigs were reported following vaccination with combined fourth-stage larvae and secreted antigens of *Trichostrongylus colubriformis* but homogenates of second-stage larvae and adult worms, without antigen collected during culture, also gave efficient protection. Preliminary results indicated that rabbits could be vaccinated with combined fourth-stage larvae and secreted antigens of *Strongyloides papillosus*.

A recent re-evaluation of vaccination against *Trichostrongylus colubriformis* in the guinea-pig has been made by Rothwell and Love (1974). In this careful study, equal quantities of homogenate protein derived from third- and fourth-stage larvae or adult worms were used to vaccinate guinea-pigs with a single dose in complete Freund's adjuvant. A highly significant level of resistance to a challenge infection was induced with 100 μg of protein from homogenates of fourth-stage or adult worms but homogenates of third-stage larvae did not

give a significant level of protection. The same amount of protein secreted by fourth-stage larvae during a 3 h period *in vitro* gave protection of the same order (> 80%) as homogenate derived from fourth-stage larvae or adult worms. These results show that functional antigens of *Trichostrongylus colubriformis* are present in much the same amounts in fourth-stage larvae and in adult worms. Evidently, in this species, functional antigens are not associated with the moulting process and there is evidence that the secretory antigens are produced by the excretory and oesophageal glands which are as well developed in fourth-stage larvae of *T. colubriformis* as in the adult worm (Ogilvie *et al.*, 1973). It is also clear from these results that secretory antigens can be obtained as readily from homogenates of fourth-stage larvae or adults as from secretions harvested from cultures *in vitro*.

Very variable results have been reported in experimental vaccination with secretions and homogenates of larval stages of *Ascaris suum*. Soulsby (1963) compared secretions in culture fluid from third-stage larvae with homogenates of larvae or mixtures of antigen from both these sources. Guinea-pigs were vaccinated with two subcutaneous injections of antigen in complete Freund's adjuvant at an interval of two weeks. Protection, judged by the numbers of larvae recovered from the lungs seven days after challenge with infective eggs was as marked with antigens in homogenized larvae as with soluble antigens in culture fluid (> 80%).

Guerrero and Silverman (1969) found that antigens secreted by third-stage *Ascaris* larvae during five days *in vitro* were more effective than extracts of the larvae for vaccinating mice. Lyophilized culture medium or French press extracts of larvae were given in two intraperitoneal doses separated by 14 days using 4% sodium alginate gel as an adjuvant. Recovery of larvae from the lungs seven days after a challenge with eggs was depressed by 74% after vaccination with secretions but by only 32–59% when extracts of larvae were used. In this study second-stage larval secretions were not effective although Crandall and Arean (1965) had found that secretions collected during 24 h maintenance of second-stage larvae in Hanks' balanced saline gave quite high levels of protection (about 75%). Guerrero and Silverman (1971) subsequently found that second-stage *Ascaris* larvae could yield effective quantities of secreted antigen but only after a period of 12 days culture *in vitro*.

It is difficult to draw any definite conclusions from these conflicting data on *Ascaris suum* but it is evident that under some experimental conditions high levels of protection can be achieved both with antigens secreted by second- or third-stage larvae and with antigens in extracts from these stages. Undoubtedly, characterization of the functional antigens involved would greatly facilitate direct comparison of these different sources of supply.

Some progress towards the characterization of the functional antigens obtained from cultures of third- or fourth-stage larvae of *Haemonchus contortus* has been reported (Ozerol and Silverman, 1969; 1970). Antigenic material secreted during the moulting process into a relatively simple protein-free culture medium was fractionated on Sephadex G-200 and two major fractions were obtained from cultures of each larval stage. In both cases, only the first peak contained antigens which reacted with antibodies in immune

sheep serum and these fractions gave 70–80% protection against challenge when used to vaccinate lambs. Further fractionation of the active material was obtained on Biogel but these sub-fractions were not tested individually. In contrast Neilson (1969, 1975) found that antigens from cultures of larval *H. contortus*, partially isolated by gel-filtration, were precipitated by antibodies in immune sheep serum but gave no protection when used to vaccinate lambs. A range of antigen concentrations from 0·05–5·0 mg was tested with and without Freund's complete adjuvant. The reasons for these wide differences in vaccination results with *Haemonchus contortus* in lambs are not apparent, but the age of the animals at vaccination may have been responsible. Attempts to develop a vaccine using X-irradiated *H. contortus* have shown that while sheep aged over six months could be protected, lambs aged 2–4 months completely failed to develop resistance to a challenge infection (Urquhart *et al.*, 1966). The lambs which Neilson (1975) tried to vaccinate were three months old but the age of the animals successfully vaccinated by Ozerol and Silverman (1970) was not given.

Reviewing immunological unresponsiveness of young animals to nematode infections Jarrett and Urquhart (1971) point out that although it is well established for *H. contortus* in lambs it is not a feature of all nematode infections. Irradiated vaccines against bovine lungworm and canine hookworm, for instance, are very effective in young animals. Immunological unresponsiveness in young livestock can present a serious problem in the development of any helminth vaccine, because under field conditions, exposure to infection can occur very soon after birth.

6. *Vaccination with isolated* Ascaris *enzymes*

In the course of studies on malic dehydrogenase in *Ascaris suum* Rhodes *et al.* (1965) showed that the partially purified enzyme could be used to stimulate the formation of specific antibodies in pigs or guinea-pigs which would inhibit the activity of the parasite enzyme but was without inhibitory effect on malic dehydrogenase from pig heart muscle. This antigenic individuality of an enzyme with the same catalytic function in parasite and host suggested that parasite malic dehydrogenase might be effective in vaccination. In fact, guinea-pigs vaccinated with three doses of partially purified enzyme in complete Freund's adjuvant were protected to some extent against a challenge with *Ascaris* eggs. There was a reduction of approximately half in the numbers of larvae recovered from the lungs in two out of three experiments (Rhodes *et al.*, 1965) but a similar attempt with vaccination of pigs was not successful. Rhodes *et al.* (1969) extended this approach by isolating a particulate aminopeptidase from the intestinal tissue of *Ascaris*. This enzyme was obtained in soluble form after treatment with proteolytic enzymes and following purification by sucrose density gradient centrifugation, it migrated as a single band in starch-gel electrophoresis. This highly purified aminopeptidase induced a moderate degree of protection against challenge when used to vaccinate guinea-pigs (Ferguson *et al.*, 1969). Similarly aldolase, from *Ascaris* body wall, purified 80-fold by a combination of salt precipitation and ion-

exchange chromatography has been used to vaccinate pigs (Mishra and Marsh, 1973). After four injections of purified aldolase in complete Freund's adjuvant at weekly intervals, the recovery of larvae from the lungs following a challenge with eggs was reduced to about one third of control values.

The partial vaccination achieved with several purified *Ascaris* enzymes suggests that these enzymes may be the functional antigens involved in the induction of acquired immunity during an infection (Mishra and Marsh, 1973). Although there is no direct eivdence that these enzymes are actually secreted by living parasites, immune serum or isolated IgG from pigs immunized by infection inhibited the activity of *Ascaris* aldolase by 98%. The other enzymes aminopeptidase and malic dehydrogenase were inhibited to a lesser extent (Mishra and Marsh, 1973). Considerable confirmatory evidence would be required before it could be accepted that one or all of these enzymes are the functional antigens involved in induction of acquired immunity but from the point of view of practical vaccination, this kind of study on isolated enzymes has considerable potential value. It may well be that parasite antigens can be isolated which are highly effective in vaccination even though they are not normally presented to the host during a natural infection.

D. CONCLUSIONS AND COMMENTS

The available evidence indicates clearly that high levels of protection against quite a wide range of nematodes can be induced by use of dead vaccines. Functional antigens, responsible for stimulating a protective immune response, have been detected in secretions from excretory and oesophageal glands in strongylid nematodes and the stichocyte secretory cells associated with the oesophagus of trichuroid nematodes. In Ascarids the functional antigens seem to be predominantly associated with larval secretions which may be involved in the moulting process although there is very little direct evidence for this view. In some strongylid and trichuroid nematodes, it has been demonstrated that functional antigens can be obtained as readily by extraction of stored secretory material from homogenized parasites as from collection of material secreted into culture medium by living nematodes.

Impressive progress towards purification of the functional antigens of *Trichostrongylus colubriformis* (Rothwell and Merritt, 1975) and *Trichinella spiralis* (Despommier and Müller, 1976) has been made and complete isolation and characterization of the functional antigens of these species can be expected in the reasonably near future. Isolation of the functional antigens will, of course, shed light on the very interesting question of the function they have for the parasite. Are they indeed enzymes involved in extra-corporeal digestion or in maintenance of the parasite in its preferred location or do they have some other unsuspected function in the economy of the organism?

From the more practical point of view, isolation of functional antigens is likely to be a critical advance. At present the only available assay for functional antigens is a direct test of their ability to stimulate a protective immune reaction against a challenge infection by vaccination of an experimental host. This procedure is time-consuming and as the variable results reported in the

last 20 years bear witness, it has resulted in very slow progress towards identification of the functional antigens. When they have been identified by this laborious process, however, it will be possible to develop rapid and sensitive biochemical or radioimmunological assays. Simplified quantitative assays for functional antigens are likely to lead to several technical advances essential for the development of practical vaccines. Most important, methods of isolation can be improved rapidly when assay of each isolated fraction no longer depends on vaccination which often involves a gap of several weeks for determination of its effectiveness. The best source of supply of functional antigen can then be examined on a properly quantitative basis; that is, whether antigen can be obtained in greater yield from culture medium or by extraction from homogenates of whole parasites. This apparently simple problem has been implicit in most investigations of vaccination against nematodes but the very variable results reported by different workers even with the same species of parasite indicate that simplification of the experimental system is essential. In some species of nematode functional antigens may be obtainable only from culture medium because they are synthesized for immediate secretion and not stored in any appreciable quantity. Similar examples are well-known in other fields, for instance the collagenase in the tail of metamorphosing tadpoles which can only be detected as a secretion from living cells (Gross and Lapiere, 1962). At present there is no clear evidence that the functional antigens of any parasitic nematode are obtainable only by culture *in vitro* and most recent investigations have shown that stored secretory antigens can be obtained readily from homogenates of whole parasites.

When the best source of functional antigen, involving either collection from culture medium or isolation from homogenized parasite tissue, has been determined for a given parasite, the supply of large numbers of organisms remains a formidable problem. At present larval stages or adult nematodes are collected from heavily infected experimental hosts. The quantities of parasites obtained in this way are quite adequate for experimental work on vaccination, such as determining the nature and source of functional antigens, and the most effective methods of vaccination. However, in development of a practical vaccine the scaling-up of this source of supply introduces considerable problems both in the number of hosts required and the labour needed for collection of parasites.

Cultivation *in vitro* seems the obvious answer to the problem of large scale supply of parasitic nematodes. In spite of considerable technical advances in methods of culturing parasitic nematodes, notably by Weinstein and later by Berntzen, no species can yet be cultured through the entire life cycle *in vitro* (reviewed by Silverman, 1965; Taylor and Baker, 1968; Leland, 1970; Silverman and Hansen, 1971; Smyth, 1976). Culture throughout the life cycle is essential because the formation of eggs is the only phase of multiplication in nematodes. Two species, *Nippostrongylus brasiliensis* and *Cooperia punctata*, have been cultured through all stages of the life cycle but the eggs produced by adult worms were infertile. If the basic problem of inducing the formation of fertile eggs could be solved, development of large scale culture methods would

be possible and it is surprising that this area of research has attracted little interest in recent years. In *Trichinella spiralis*, which is ovoviviparous, living larvae have been produced by adult worms grown from the larval stage *in vitro* but continuous culture has not been achieved. Encouraging progress has been made in large-scale continuous culture of free-living nematodes and simplified media have been developed (Buecher and Hansen, 1971; Van Fleteren, 1976). Some cross-reacting antigens have been detected in the free-living nematode *Caenorhabditis briggsae* and the parasite *Haemonchus contortus* (Jakstys and Silverman, 1969) but there have been no reports of vaccination against parasitic nematodes with antigens of free-living forms.

1. *Prospects for vaccines*

Vaccination against the gastrointestinal nematodes of sheep and cattle would be of value even in the developed countries where reliance is currently placed on the prophylactic use of drugs. In the developing regions, vaccines, providing they could be produced at reasonable cost, would be of even greater advantage. Ideally, a mixture of vaccines acting against several gastrointestinal nematodes would be most valuable but a combined vaccine against the two major parasites of sheep, *Trichostrongylus colubriformis* and *Haemonchus contortus*, would be very useful (Mulligan, 1975). Experimental vaccination against *T. colubriformis* has been demonstrated in guinea-pigs but so far there have not been reports of vaccination of sheep. Successful vaccination of sheep against *H. contortus* has been reported (Ozerol and Silverman, 1970) but experience with a live irradiated vaccine against this parasite shows that protection of young lambs cannot be obtained (reviewed by Jarrett and Urquhart, 1971). The exposure of lambs to nematode infections immediately after birth, under field conditions, means that immunological unresponsiveness of the young animals could be a determining factor preventing practical use of vaccines. However, as Jarrett and Urquhart have pointed out lambs respond well to some nematode antigens such as the live irradiated *Dictyocaulus* vaccine and dead vaccines based on antigens secreted by larval cestodes are very effective in young lambs. These examples of successful vaccination of lambs show that unresponsiveness to *H. contortus* is not a general phenonenon and further work is clearly required to determine why functional antigens of *H. contortus* fail to immunize young lambs. In the model system, *Nippostrongylus brasiliensis* in the rat, there is evidence that immunological unresponsiveness of the young host to an infection is due to failure of the cell-mediated second step of the immune response although normal antibody formation occurs (reviewed by Ogilvie and Love, 1974).

The prospects for development of a dead vaccine against a human nematode disease are brightest for *Trichinella spiralis* which is still surprisingly common in the USA and Europe. In this case the aim will be to prevent human infection from infected pork by widespread vaccination of pigs. Antigens extracted from larval worms have been shown to be highly effective in experimental vaccination of mice (Despommier *et al.*, 1977). It is possible that sufficient numbers of larvae may be obtained from the musculature of pigs, given very heavy

experimental infections, to carry out a limited vaccination programme. However, the very large numbers of larvae required for large scale vaccination of pigs is likely to involve continuous culture of the nematode. *T. spiralis* has been cultured from infective larvae to fertile adult worms (Berntzen, 1965) and further development of the system to allow continuous culture should not prove an insurmountable problem.

The prospects for vaccines against the most widespread and serious nematode diseases of man are much less encouraging. Infections with *Ascaris* and hookworm still affect about 1000 million people in the developing countries. Migrating larvae of *Ascaris* may cause severe pulmonary symptoms, generalized allergic reactions occur and large numbers of adult worms may cause mechanical obstruction of the intestinal tract. In hookworm disease, due to *Ancylostoma* or *Necator* little overt pathology due to the infection is recognized but there is a long-term debilitating effect from continuous loss of blood caused by the feeding worms. Vaccination against *Ascaris* with secreted larval antigens has been reasonably successful in experimental animals. Comparable work has not been carried out with *Ancylostoma* or *Necator* because there is, as yet, no suitable laboratory host available, in spite of valuable attempts to adapt *Necator* to the hamster (Sen, 1972; Ogilvie *et al.*, 1975). On the basis of the development of a successful irradiated vaccine against the canine hookworm, Miller (1975) has suggested a similar approach to control of human hookworm disease. Without continuous culture, the supply of larvae for a live vaccine or extraction of antigen, is likely to be a limiting factor and research is urgently needed in this area.

Among the filarial infections of man, which affect at least 270 million people in tropical regions, *Wuchereria bancrofti* which causes elephantiasis and hydrocoel and *Onchocerca volvulus*, the agent of river blindness, are the most damaging species. Vaccines would be a very valuable adjunct to drug therapy and control of the insect vectors for filarial diseases in general (WHO, 1974a). There is a more urgent need for a vaccine against *Onchocerca* because no effective drug treatment is available. The main factor limiting the progress of research is the absence of suitable laboratory hosts for *Onchocerca* and *Wuchereria*. So far, the few attempts to vaccinate with dead worm material, against species of filariae for which experimental hosts are available, have been quite unsuccessful but secreted antigens have hardly been tested at all (reviewed by Denham and McGreevy, 1977). Results with live irradiated vaccines have been encouraging in some cases. Again, the supply of parasites for either live or dead vaccines will certainly require continuous culture of filarial worms and research in this neglected area is greatly needed.

III. CESTODES

A. INTRODUCTION

The cestodes are all obligate parasites with a complex life cycle involving development in at least two hosts, a definitive host in which the adult tapeworm produces eggs and one or more intermediate hosts in which the larval

stages develop. Serious effects on livestock and man are almost exclusively associated with the larval cystic stages of a number of taeniid cestodes. The cysts of *Taenia ovis* in the musculature of sheep lead to the rejection of substantial quantities of meat in Australasia. The adult tapeworms develop in dogs which have access to infected meat. Adult *Echinococcus granulosus* also occurs mainly in dogs and the larval stage, the hydatid cyst, which develops primarily in the liver of sheep and cattle can cause considerable economic loss. However, the major significance of this parasite lies in the fact that the cystic stage can develop in man following accidental ingestion of eggs voided by infected dogs. Cysts developing in the liver and sometimes the brain can be fatal and although the incidence of infection is relatively low (possibly in the region of 100 000 in Australia and South America) an average of two operations per day for removal of hydatid cyst, which is the only treatment available, has been reported in Australasia. Cysticercosis in cattle, due to *T. saginata* is very widespread in Africa and partly accounts for the continued failure to develop exports of meat. Adult *T. saginata* occurs only in man and Stoll (1947) estimated that there were 39 million infections mainly in Africa and the USSR. Other tapeworm infections of man have a much more restricted distribution. Three million *T. solium* infections with a cystic stage in pigs occur mainly in Asia and *Diphyllobothrium latum*, the larvae of which are ingested with infected fish, may infect another three million around the Baltic in northern USSR and Asia. In all there are probably about 72 million human tapeworm infections (Stoll, 1947).

Studies on the development of immunity in the intermediate host (reviewed by Weinmann, 1970; Gemmell and Macnamara 1972; Gemmell, 1976) have shown clearly that a strong immune response is acquired to the larval cysticercoid stage of many cestodes. There is evidence that antibody may act either in preventing establishment of the oncosphere emerging from the egg or alternatively during the early stages of development of the oncosphere. However, mature cysts seem to become relatively insusceptible to immune destruction. Cell-mediated reactions may also be involved in the effective immune reaction against cestode larvae but relatively little work has yet been done in this area. Much less effective immune reactions are induced by the adult stages of tapeworms which predominantly inhabit the intestinal lumen. Many workers have doubted whether a primary infection stimulates any protection against subsequent challenge infections but recent evidence (reviewed by Gemmell, 1976) suggests that some degree of resistance develops which has an immunological basis. In view of the high levels of immunity to reinfection with larval stages of cestodes acquired by intermediate hosts, it is perhaps not surprising that vaccination against these larval stages has been much more successful than vaccination against adult tapeworms.

B. VACCINATION AGAINST LARVAL CESTODES

A number of quite early attempts were made to vaccinate laboratory hosts against the larval cysticercoid stages of *Taenia taeniaeformis* and *T. pisiformis*.

Miller (1931a,b, 1932) gave rats repeated injections of powdered antigen prepared from dried cysts of *T. taeniaeformis* and obtained fairly high levels of protection against infection with living eggs of the parasite. Using the same model system, Campbell (1936) produced absolute immunity to infection with *T. taeniaeformis* by giving six intraperitoneal injections of dried or fresh cyst material. In these interesting experiments Campbell distinguished between immunity acting to prevent establishment of the oncosphere, the infective stage which hatches from the egg and destruction of the cyst at a later stage of development. Crude antigens prepared from cysts prevented 75% of oncospheres from establishing in the host and destroyed the remainder during development in the liver. In spite of these promising results with *T. taeniaeformis*, similar vaccination against *T. pisiformis* in rabbits was much less successful. Even after six injections of fresh or dried cyst material by a variety of routes, rabbits were only marginally protected (Miller and Kerr, 1932). Later Kerr (1935) claimed better results using fresh or dried adult *T. pisiformis* material to vaccinate rabbits against the cysticercoid stage but the levels of protection achieved were very variable. Although the relative success of vaccination against *T. taeniaeformis* with crude cyst and adult worm material might have been expected to lead to refinement of this system by isolation of the effective, "functional" antigens, interest in empirical vaccination studies languished until the 1960s.

1. *Vaccination with secreted antigens*

Following the demonstration that highly effective protection could be achieved against the nematode lungworm, *Dictyocaulus viviparus* in cattle with a living vaccine based on X-irradiated larvae (Jarrett *et al.*, 1960) this approach was extended to cysticercosis in cattle caused by *T. saginata* (Urquhart, 1961). Eggs of *T. saginata* were X-irradiated and fed to calves which developed a substantial level of resistance to a subsequent challenge with normal eggs. These promising initial results were not taken further mainly because adult *T. saginata* only develops in man and this imposes almost insuperable problems in the collection of large numbers of eggs for irradiation. Successful vaccination with an irradiated larval cestode preparation however, led to other attempts to vaccinate against cysticercosis with living organisms.

In a series of investigations on cysticercosis in sheep caused by *T. ovis, T. hydatigena* and *Echinoccocus granulosus*, Gemmell (1962a, 1964a,b, 1965a,b, 1966) showed that living eggs or oncospheres hatched from them when injected intramuscularly could stimulate quite high levels of resistance.

In an ectopic site in the muscle, eggs or activated oncospheres are able to develop to a very limited extent and this technique effectively allows the host to be exposed to a truncated living infection which is sufficient to provoke resistance to challenge.

Some cross-immunity between the three species was observed in lambs vaccinated with activated oncospheres but the rabbit cysticercus *T. pisiformis* did not cross-protect. Eggs or oncospheres killed by freezing failed to induce any resistance in lambs and Gemmell concluded that the functional antigens

stimulating resistance were produced by the living organisms. Heath (1973) found that larvae of *T. pisiformis* which had undergone up to 15 days partial development in rabbits or in an in-vitro culture system could stimulate very high levels of resistance to challenge in rabbits following subcutaneous implantation. Larvae older than 15 days stimulated much lower levels of immunity and subcutaneous implantation of fully developed 42 day old cysticerci did not result in any protective response. These results showed that the functional antigens are produced only by younger larvae but as Heath (1973) pointed out, since many larvae die and disintegrate after implantation, this kind of experiment does not necessarily imply that the functional antigens are secretory products of the living organism. More direct evidence implicating secreted antigens was obtained by Rickard and Bell (1971a) who implanted oncospheres of *T. ovis* and *T. taeniaeformis* which were contained within millipore diffusion chambers into the peritoneal cavity of lambs or rats. Complete immunity to challenge with eggs of *T. ovis* was obtained when the diffusion chambers had been *in situ* for only one week but this level of resistance to *T. taeniaeformis* did not develop even after three weeks implantation. Only a small proportion of the larvae in the diffusion chambers survived to the end of the experiments and "somatic" antigens from disintegrating organisms could have contributed to the immunizing effect.

Although secreted antigens collected from living nematodes during cultivation *in vitro* had been implicated as functional antigens by the early 1960s similar work with cestodes seems to have been greatly delayed. Culture techniques for larval taeniid cestodes were not introduced until Heath and Smyth (1970) showed that artificially hatched oncospheres of *T. ovis, T. hydatigena* and *T. pisiformis* could undergo considerable development *in vitro*. Up to this time cultivation studies on cestodes had concentrated largely on the stages of development from cysticercoid to adult and, with the exception of *Echinococcus granulosus* on non-taeniid cestodes (reviews by Silverman, 1965; Silverman and Hansen 1971; Smyth, 1969).

Using the simple culture system developed by Heath and Smyth (1970), Rickard and Bell (1971b) vaccinated lambs against *T. ovis* with antigens collected during culture *in vitro*. Large numbers of activated oncospheres were cultured for eight days and concentrated medium derived from about 20 000 organisms was given in a single intramuscular injection to each lamb after emulsification with Freund's complete adjuvant. Complete resistance was obtained to a challenge with viable eggs given five weeks later. Vaccination with *T. hydatigena* antigens derived from culture medium also gave a very high level of protection against challenge with *T. ovis*.

The oncospheres cultured *in vitro* in these experiments were obtained from eggs harvested from adult *T. ovis* or *T. hydatigena* grown in beagle dogs. It remains to be determined whether the number of eggs used for a single vaccination can be reduced to a low enough level for this source of antigen to be used on a practical scale.

Culture fluid antigens have given a high level of protection in calves against cysticercosis caused by *T. saginata* (Rickard and Adolph, 1976). The eggs of *T. saginata* can be obtained only from human infections and the difficulty

of obtaining large numbers for culture encouraged an attempt at cross-protection. Secreted antigens derived from cultures of *T. ovis* and *T. hydatigena* eggs obtained from dogs stimulated only about 55% resistance to a heterologous challenge with *T. saginata* compared with the almost complete protection afforded by homologous vaccination.

Secreted antigens from *in-vitro* cultures of larval cestodes have also been used with success to vaccinate against several species, which although of no economic significance, provide very valuable models for experimental investigation. High levels of resistance but not complete protection of rabbits to *T. pisiformis* have been obtained using such secreted antigens (Heath, 1973, 1976; Rickard and Outteridge, 1974). Kowalski and Thorson (1972) found that vaccination of mice with antigens secreted by tetrathyridia of *Mesocestoides corti* over a very short culture period of 24 h gave only a 60% level of protection against a challenge. A similarly short period of culture using *Taenia taeniaeformis* allowed collection of secreted antigens which gave 90–100% protection against a homologous challenge in rats (Kwa and Liew, 1977).

2. *Secreted versus somatic antigens*

Although material secreted into culture medium by a range of larval cestodes has been shown to contain functional antigens which are capable of stimulating high levels of protection, it cannot be assumed that this represents the most efficient source of antigen for vaccination. An illustration of this point is afforded by recent work on *T. saginata*. Complete protection can be obtained by vaccination of calves with a soluble extract of "somatic" antigens derived from adult worm tissues (Gallie and Sewell, 1976).

The antigens extracted from adult *T. saginata* were given in a series of six intramuscular injections in complete Freund's adjuvant whereas the culture fluid antigens were given only once in incomplete Freund's. It would clearly be necessary to carry out direct comparison of the secreted and somatic antigens of *T. saginata* in a single series of experiments in order to determine the most efficient supply of antigen for vaccination.

Attempts have been made to compare secreted and somatic antigens directly using larval cestode infections in laboratory hosts but for various reasons most of the results have been inconclusive. In preliminary experiments with *Mesocestoides corti*, antigens secreted by the larval tetrathyridia stage maintained for 24 h in balanced saline were compared with soluble antigens extracted from the larvae (Kowalski and Thorson, 1972).

After a series of injections of antigens from these sources, mice were challenged by oral or intraperitoneal administration of infective larvae. Vaccination with secreted antigens caused a reduction of 60% in the number of tetrathyridia which survived but somatic antigens gave only a 30% reduction following oral challenge and no effect against intraperitoneal implants. The amounts of secreted and somatic antigens used in these experiments were not measured.

Heath (1976) compared the relative effectiveness for vaccinating rabbits of

antigens secreted by larval *T. pisiformis* with somatic antigens in whole larvae which had been killed by freezing. In order to obtain secreted antigens free of serum macromolecules in the culture medium, larvae were allowed to develop in full medium for ten days; after this period, they are able to survive without a serum component in the medium. Ten day old larvae were transferred to fresh medium which had been previously filtered through a molecular sieve membrane to remove molecules above 10 000 M.W. After concentration, secreted antigens collected in this way and adsorbed on to aluminium phosphate gave 88% protection in rabbits against challenge following a single vaccination.

Whole larvae administered without adsorbent stimulated 52% protection. These experiments do not indicate whether the secreted or somatic antigens were superior because the amounts administered were not measured and adjuvant was employed only for the secreted antigens. Nevertheless, some functional antigen is evidently available from both sources although, without further analysis, it is not clear whether the antigens involved are identical.

3. *Isolation of functional antigens*

The problems involved in comparing the effectiveness of secreted and somatic antigens have been resolved in very recent work on *Taenia taeniaeformis* (Kwa and Liew, 1977) by isolation of the functional antigens. Secreted antigens collected from larval organisms maintained for 24 h in a tissue culture medium without serum were first compared with crude soluble somatic antigens extracted from homogenized larvae. Rats were vaccinated with 1 mg total protein of either secreted or somatic antigens in complete Freund's adjuvant. Challenge with oncospheres at various intervals after vaccination showed that very high levels of protection had developed. Fractionation of secreted and somatic antigens by column chromatography on Sephadex G200 gave two distinct and several minor peaks for the somatic antigens. The secreted antigens chromatographed in two main peaks which coincided with the major components of the somatic antigens. Analysis of individual fractions by double immunodiffusion on Ouchterlony gels using antiserum from infected rats, showed that the peak antigenic activity occurred in the same fraction (number 62) in both preparations. Only one protein band with an estimated molecular weight of 140 000 was detected by polyacrylamide gel electrophoresis. This protein occurred in fraction 62 in both cases. Vaccination of rats with graded amounts of this purified antigen in complete Freund's adjuvant showed full protection at the highest dosage of 50 μg with about 90% protection at the 10 μg level and 70% at 1 μg.

This very interesting investigation demonstrates clearly that the same highly effective functional antigen can be obtained from secreted material and from somatic extracts. Estimation of the relative amount of functional antigen in somatic and secreted material suggested that the antigen accounts for 7–8% of the total material in both cases. The authors conclude that it will be simpler and more efficient to purify functional antigen from somatic material than to collect secretions from in-vitro culture.

Isolation and direct comparison of the functional antigens in culture fluid and somatic extracts of other larval cestodes are clearly needed and some progress towards isolation has been made with *T. pisiformis*. Serum-free secreted antigens collected from ten day old *T. pisiformis* larvae and shown to vaccinate rabbits, contained six protein components on analysis using polyacrylamide electrophoresis (Heath, 1976). Amino-acid analysis of the crude combined antigens revealed that antigens collected from cultures which had been gassed with oxygen-free nitrogen contained low levels of two aromatic amino-acids, cysteine and methionine which could not be detected in similar antigens collected from cultures maintained under aerobic conditions. Moreover, only the unoxidized antigens were capable of stimulating high levels of protective immunity in rabbits. Loss of antigenicity due to oxidation of secreted antigens clearly has a bearing on measurement of the amount of functional antigen produced and this factor will have to be examined in the preparation of secreted antigens in other systems.

4. *Immunological correlates of vaccination*

At present the only method available for measuring the effectiveness of a given antigen preparation in vaccination is a direct test of its ability to stimulate a protective immune response to a challenge infection. Vaccination experiments with even the simplest experimental models such as *T. taeniaeformis* in the rat or *T. pisiformis* in the rabbit are relatively time-consuming and in vaccination of livestock the time and effort involved can be daunting. Correlation of effective vaccination with a simple test for specific antibody or an intradermal test using purified antigen would be a very valuable advance. Some work towards this objective has been carried out but so far no simple correlate has emerged.

Antibodies to *T. pisiformis* culture fluid antigens could not be detected by standard serological tests in sera from rabbits successfully vaccinated with these antigens (Rickard and Outteridge, 1974). However, an intradermal test with crude culture antigens gave a strong delayed-type hypersensitivity (DTH) reaction in naturally infected rabbits. After vaccination with culture fluid antigens, rabbits showed a definite though lower level of DTH reaction. To determine whether this intradermal DTH reaction could be elicited with the functional antigen responsible for vaccination, Rickard and Katyiar (1976) fractionated crude antigens collected from cultures of *T. pisiformis*. Column chromatography on Sephadex G200 gave four fractions one of which gave a strong DTH reaction in infected rabbits.

Further fractionation on DEAE ion-exchange sephadex yielded a fraction which gave as strong a DTH reaction as the starting material but contained only 5% of the original protein. Unfortunately this purified fraction failed to vaccinate rabbits against challenge. Evidently in this system the antigen responsible for the DTH reaction is not the functional antigen which stimulates protective immunity in vaccination.

Vaccination of rats against larval *T. taeniaeformis* with both secreted and somatic antigen preparations stimulated immediate-type and delayed-type

hypersensitivity reactions to similar levels (Kwa and Liew, 1977). Both IgG and IgM to crude secreted and somatic antigens were detected from the second week after vaccination. Levels of IgG were considerably higher than levels of IgM. The activity of the functional antigen purified from secreted and somatic material in stimulating specific antibody or the immediate or delayed dermal hypersensitivity reactions remains to be determined. However since isolation of the functional antigen has been achieved in this system, correlation with a simple test seems very likely.

5. *Vaccination in the field*

A field trial of vaccination of lambs against cysticercosis caused by *T. ovis* using secreted antigens collected during culture of oncospheres *in vitro* has been carried out by Rickard *et al.* (1976). Under field conditions, lambs grazing on pastures contaminated with the eggs of *T. ovis* voided by dogs may ingest very variable numbers of eggs each day.

To determine whether vaccinated lambs can resist a variable natural challenge, 30 lambs obtained from a *T. ovis* free source were vaccinated with a single dose of secreted antigens in incomplete Freund's adjuvant and introduced four weeks later to experimental plots contaminated with three different levels of *T. ovis* eggs. A control group of the same size sham-vaccinated with culture medium in adjuvant was similarly exposed to infection. The vaccinated lambs showed strong resistance even to the highest levels of challenge although the complete immunity seen in lambs given a single standardized challenge (Rickard and Bell 1971b) was not observed even in lambs exposed to a low level infection. Comparison of vaccinated lambs with naturally infected sheep showed that vaccination gave more efficient protection than a living infection. However, the vaccinated lambs were not able to destroy the few cysticerci which became established, at a later stage of development whereas in naturally infected sheep, a high proportion of established cysts were degenerating. Evidently vaccination in this system must aim at total prevention of infection.

An important practical problem in field vaccination of livestock is that young lambs and calves are exposed to infection from pastures contaminated with eggs from the first few days of life. It is hardly practicable to vaccinate at this age and, in any case, there is some evidence that young lambs do not have full immunological competence towards cestode antigens (Gemmell *et al.*, 1968) a problem which has also been encountered with vaccination of livestock against nematode infections (Jarrett and Urquhart, 1971). Transfer of immunity from a naturally infected mother to offspring via colostrum is known to occur in many diseases and if this was a feature of immunity to cestode larvae, the young animal might be naturally protected at least during the first few weeks of life allowing time for effective vaccination to be carried out.

Cows naturally infected with *T. saginata* cysticerci did not apparently transfer any detectable degree of resistance to their calves (Urquhart, 1961). Similarly, lambs born to ewes with natural *T. hydatigena* infections were not protected against experimental infection (Gemmell *et al.*, 1969). However,

ewes which had been hyperimmunized during pregnancy with intramuscular injections of living oncospheres were able to transfer some protection to lambs which lasted for about one month after birth. In contrast lambs from ewes infected with *T. ovis* showed almost complete protection against experimental infection for about nine weeks and vaccination with culture fluid antigens during this period induced complete subsequent protection (Rickard and Arundel, 1974). The high level of protection transferred by naturally infected ewes in these experiments prevented assessment of whether immunity induced by vaccination of ewes could also be transferred to lambs. Further studies are clearly required in this area but on present evidence, it seems likely that if high levels of resistance to *T. ovis* or *T. hydatigena* are established in a flock, a satisfactory level of protection will be transferred to lambs allowing a long enough period for effective vaccination.

C. VACCINATION AGAINST ADULT CESTODES

Nearly all attempts to vaccinate against adult tapeworms have concentrated on *Echinococcus granulosus* in the dog because eggs voided by dogs which normally complete the life cycle by infecting sheep and cattle, can be accidentally ingested by man. Development of the larval stage, the hydatid cyst, in man usually necessitates surgical removal because there is no effective drug treatment. Damage to the hydatid cyst during surgery often releases secondary cysts or protoscolices which develop in other sites often with a fatal outcome.

Initial attempts to vaccinate dogs against *E. granulosus* seem to have been moderately successful. Turner *et al.* (1933) using crude antigen prepared from dried hydatid cyst walls or dried protoscolices gave dogs a series of five intramuscular injections at intervals of 3–5 days. Following challenge with living protoscolices 6–15 days later, eight vaccinated dogs had between 0–6 adult worms whereas controls had enormous numbers of worms. In a much larger trial using 106 dogs, only about half the vaccinated animals resisted an infection compared with a 95% infection level in control dogs (Turner *et al.*, 1936). Partial protection of dogs by vaccination with dried material from protoscolices has also been reported (Matov and Vasilev, 1955) and extracts of cyst walls, protoscolices and even hydatid fluid have given some degree of vaccination (Forsek and Rukavina, 1959; De Rosa *et al.*, 1974).

In a careful study Gemmell (1962b) showed that vaccination with crude antigen prepared from lyophilized protoscolices or adult worms administered once in an oil-based adjuvant or five times without adjuvant could significantly reduce the number of adult worms developing from a challenge infection. The mean reduction for 16 vaccinated dogs was about 65% and there was some evidence that adult worm antigens were more effective than the larval antigens obtained from protoscolices. The worms which did establish in vaccinated dogs had fewer and less well developed terminal and sub-terminal segments than those from control dogs. Developing eggs were entirely absent from these undersized segments and although it was not claimed that the worms were completely sterile, a decisive effect on egg production seems to have been produced by vaccination.

Following the demonstrations that functional antigens capable of stimulating high levels of immunity against larval stages of cestodes are present in material secreted into culture media, Herd *et al.* (1975) have examined the effect of secretions from adult worms in protecting dogs against *E. granulosus*. In order to collect secretions free from serum components, worms were recovered from dogs at a late stage of development and maintained in a protein-free medium based on the growth medium developed by Smyth (1967). After concentration, secreted material was administered to six dogs in 5 mg doses with complete Freund's adjuvant, four times over a total period of 17 weeks. The number of worms which developed following challenge with protoscolices was not significantly reduced but the proportion of worms containing eggs was ten times lower in vaccinated dogs. Thirteen protein components were detected in the secreted material but serum from vaccinated dogs contained antibodies against only three of them.

Although the evidence so far available indicates that vaccination of dogs against adult *E. granulosus* does not result in high levels of protection, the blocking of egg production is a very interesting result. However, in the most recent work on this system (Herd, 1977) inhibited development and failure of egg production was observed in all the worms from a number of control dogs. The controls had received the adjuvants *B. pertussis* and complete Freund's without secreted adult worm antigens. It is not clear whether the adjuvants were responsible for the effect on the parasites or whether some dogs show high levels of natural resistance and further work to clarify this question is clearly required. The high levels of immunity against some larval cestodes induced by vaccination with secreted larval antigens demonstrates that these are highly effective immunogens and it is of considerable interest to know whether the immunity which they confer is also effective against adult tapeworms. Vaccination of young beagle dogs with antigens secreted into culture medium by oncospheres of *T. pisiformis* completely failed to prevent the development of adult *T. pisiformis* (Rickard, personal communication) although rabbits vaccinated with antigens collected in this way are highly resistant to infection with larval *T. pisiformis* (Rickard and Outteridge, 1974). These results suggest that the functional antigens of the larval stages of *T. pisiformis* are not present as target antigens in adult worms.

D. NON-SPECIFIC RESISTANCE TO CESTODES

Bacillus Calmette-Guerin (BCG) an attenuated strain of *Mycobacterium bovis* used to vaccinate against human tuberculosis, has been employed extensively to stimulate non-specific resistance against neoplasms in experimental animals and man (review by Laucius *et al.*, 1974). The larval stage of *Echinococcus multilocularis* has an interesting, if superficial resemblance to neoplasia because the individual cysts bud and metastasize to new sites in the intermediate host. On the basis of this resemblance Rau and Tanner (1975) pre-treated cotton rats with BCG one week before an intraperitoneal challenge with protoscolices of *E. multilocularis*. A dose of $26{\cdot}4 \times 10^6$ viable organisms of BCG given intraperitoneally one week before challenge resulted in a

substantial reduction in the number of cysts developing from the challenge. Control rats had a mean count of 40·5 cysts which was reduced to a mean of 0·8 in treated rats with two of four treated animals showing complete protection. A similar dose of BCG two weeks after infection severely suppressed the numbers of cysts which developed but had no effect on the total weight of cyst material. Apparently treatment with BCG before infection affects both the growth of cysts and their capacity to divide but BCG given two weeks after infection only affects division.

The analogy between the neoplastic-like development of *E. multilocularis* and the effect of BCG treatment has little real relevance because BCG treatment has now been tested successfully against *E. granulosus* which does not have cysts which divide and metastasize to new locations. Protoscolices of *E. granulosus* were given intraperitoneally to Mongolian gerbils, a suitable experimental intermediate host, seven days after an intraperitoneal injection of $26{\cdot}5 \times 10^6$ viable BCG organisms (Thompson, 1976). In 14 treated gerbils, all development of secondary cysts was inhibited but three of the treated animals died and all showed small intraperitoneal granulomatous lesions. The marked effect of BCG on developing cysts of *E. granulosus* and *E. multilocularis* is likely to be due to non-specific activation of macrophages which is considered to be the main basis of its activity against neoplasms (Laucius *et al.*, 1974). Treatment with BCG may have a similar effect against other larval cestodes and it is interesting to note that activity against developing *Schistosoma mansoni* has also been reported.

So far protection against the larval stages of *Echinococcus* sp. with BCG has been of very short duration and investigation of long term protection,which would be essential for prophylaxis, is needed.

E. CONCLUSIONS AND COMMENTS

Impressive progress has been made in the last few years towards effective vaccination against cysticercosis in sheep and cattle caused by the larval stages of *Taenia ovis, T. hydatigena* and *T. saginata*. High levels of protection and in some cases complete immunity, have been induced with a single vaccination dose of antigens collected during *in-vitro* culture of the early stages of development of the larval organisms. The functional antigens collected in this way are likely to be secretory products of the young parasite but until they have been isolated and characterized their true nature will remain in doubt. They may well be enzymes secreted by the oncosphere either to aid initial penetration or to assist nutrition of the developing larva by digestion of surrounding tissue. At present, however, there is practically no information on the nature of the functional antigens of larval cestodes and they may play a quite unsuspected role in the physiology of the parasite.

Purification of the functional antigens of one larval cestode, *T. taeniaeformis*, has very recently been achieved and isolation of the functional antigens of *T. pisiformis* is likely in the near future. Isolation of functional antigens and the development of simple assays for their quantitative measurement can be expected to result in greatly improved methods of purification. The

development of such assays will allow precise determination of the practical question of whether functional antigens can be more efficiently collected by culturing larval organisms *in vitro* or whether they can be obtained in better yield from larval or adult cestode tissue.

On the basis of the small amount of information now available, it seems possible that functional antigens may be obtained more efficiently from larval or even adult tissues than from in-vitro cultures. At present the only assay for measuring the level of resistance induced by vaccination is by following the fate of a challenge infection. Even in the most suitable experimental models such as *T. pisiformis* in the rabbit or *T. taeniaeformis* in the rat, this is a time-consuming procedure and a simple, rapid test for measuring resistance in vaccinated hosts is badly needed. So far, attempts to correlate the presence of a particular class of antibody or intradermal reaction to crude antigens with the level of resistance have not been successful but the use of purified functional antigens can be expected to lead to the development of simple assays which should greatly speed up the testing of potential vaccines.

1. *Prospects for vaccination*

Although vaccination of livestock against cysticercosis is clearly feasible and the solution of many of the practical difficulties is within sight, commercial development of dead vaccines is unlikely to proceed until the basic problem of an adequate supply of functional antigen has been overcome. At present crude functional antigens are collected from cultures of large numbers of eggs obtained from adult tapeworms grown in dogs. Scaling-up this source of production even on the most favourable estimates would involve the use of relatively large numbers of dogs for the many millions of vaccine doses required. Apart from the ethical problems that the use of this host would raise in many countries, the amount of labour involved would result in an excessively costly product for the agricultural market.

Although it may well prove possible to obtain functional antigens in better yield by direct isolation from larval or adult worm tissues, the problem of obtaining sufficient quantities of larval or adult tapeworms remains. The most obvious solution would be continuous mass-culture of these cestodes through the complete life cycle to take full advantage of the enormous capacity of the adult tapeworm to produce eggs. Techniques for cultivation of cestodes *in vitro* are well advanced and, in fact, one species, *Hymenolepis nana* has been cultured through the complete life cycle (reviews by Taylor and Baker, 1968; Clegg and Smyth, 1968; Silverman and Hansen, 1971; Smyth, 1976). There is no doubt that the precise requirements for cultivation of different species of cestode vary greatly but successful cultivation of the taeniid worms for which vaccines are available should be feasible given sufficient research effort. Cultivation of the larval stages from the egg to the cysticercus has been largely achieved but the cysticercus to adult phase is likely to present special difficulty due to the size of the adult worms (Smyth, 1976).

So far, the results reported for vaccination against adult cestodes have been much less promising than those obtained for larval stages. However, control

of the adult stage of *Echinococcus granulosus* in dogs is highly desirable in view of the serious consequences of accidental human infection with the larval hydatid cyst. Widespread vaccination of all dogs likely to be exposed to infection is almost certainly the ideal approach rather than attempts to eradicate hydatid cyst from livestock by vaccination. The limited progress made in vaccination against the adult worm urgently needs consolidation and improvement.

IV. TREMATODES

A. INTRODUCTION

The digenetic trematodes of most serious importance to the health of man are the schistosomes or blood flukes, which are estimated to affect some 200 million people in 70 countries (WHO, 1976a). *Schistosoma mansoni* and *S. haematobium* are endemic in Africa and parts of the middle east. *S. mansoni* has also become established in Central and South America and *S. japonicum* occurs mainly in the Philippines, Japan and China. The schistosomes are unusual in their location in the blood vessels draining the intestine or bladder: most digenetic trematodes are parasites of the intestinal tract and associated organs of vertebrates. Few other trematodes are at all common in man but *Clonorchis sinensis,* which lives in the bile ducts, and *Fasciolopsis buski,* have been estimated to affect about 29 million people in Asia (Stoll, 1947). *Fasciola hepatica,* the liver-fluke, occurs quite rarely in man but seriously affects production in sheep and cattle in many parts of the world. All the digenetic trematodes have complex life cycles involving at least one intermediate host. The first intermediate host is, with rare exceptions, a snail in which the parasite multiplies asexually producing large numbers of larval stages. Infection of the definitive host generally occurs by ingestion of an encysted larval stage but infective schistosome larvae, the cercariae, penetrate directly through the skin.

Studies on immunity to trematodes have been largely confined to schistosomiasis (reviewed by Lewert 1970, Smithers and Terry, 1969, 1976; Smithers, 1976). One of the striking features of this disease, as of many other helminth infections, is the longevity of the adult worms which survive for several years in man. Many of the eggs produced by the adult parasite fail to pass through the intestinal or bladder wall and in these tissues and the liver they stimulate a cell-mediated inflammatory response. The resulting granulomata are responsible for the pathology associated with the disease but the immune response to the egg plays no part in resistance to reinfection. Experimental work with a range of animal hosts has led to general acceptance that some degree of immunity develops which is primarily stimulated by the adult schistosome. Paradoxically the immune response acts to limit reinfection by cercariae but has little effect on established adult worms. Very young schistosomes, called schistosomula, are vulnerable to immune attack but this susceptibility is rapidly lost during the first few days. At the same time host molecules, mainly derived from the red cells, are adsorbed on to the surface of

the young worm and these may block attachment of antibodies to surface antigens. Passive transfer experiments in mice and rats have implicated antibody in the mechanism of immune damage to schistosomula but only partial transfer of protection has been achieved. In-vitro assays have demonstrated direct complement-mediated antibody damage to the surface of schistosomula and several mechanisms involving cooperation between antibody and non-sensitized cells (reviewed by Capron *et al.*, 1976). Neutrophil polymorphs can damage schistosomula in the presence of IgG_2 and complement. Eosinophils are able to cooperate with some class of IgG in the absence of complement. Macrophages may act in conjunction with IgG_1 opsonizing antibody and macrophages activated by complexes of IgE and schistosome antigen can damage the surface of schistosomula. Of these antibody dependent cell-mediated mechanisms shown to be effective *in vitro* only the eosinophil has been demonstrated to play a role *in vivo*, by experiments involving depletion of eosinophils in immune mice with highly specific antiserum.

Immunity to *Fasciola hepatica* has been detected in cattle but there is no evidence that sheep develop resistance to this parasite (reviewed by Smithers, 1976). In experimental infections of rats or mice a high level of immunity develops which limits reinfection, as in schistosomiasis, without affecting established adult worms. Passive transfer of serum from infected cattle or sheep can limit infection in rats and adoptive transfer of immunity with sensitized lymphocytes has been demonstrated but the mechanism of immunity has not been determined.

By comparison with the relative success of vaccination against some nematodes and larval cestode infections, little progress has been made with dead vaccines against schistosomes or *Fasciola*. However, schistosomiasis is classed as a major disease of public health importance and development of an effective vaccine is greatly needed (WHO, 1974b).

B. VACCINATION AGAINST SCHISTOSOMES WITH HOMOGENATES

The earliest attempts to vaccinate against schistosomes were made by Japanese workers using the oriental species *Schistosoma japonicum* in dogs. Ozawa (1930) gave several dogs a single intravenous injection of adult worms or cercariae homogenized in saline. Although all the dogs became infected following a challenge with living cercariae, Ozawa considered the symptoms of disease to be milder than in control dogs given the same challenge. More objectively, fewer worms could be recovered from vaccinated dogs and they were stunted by comparison with control worms. Very similar results were reported by Kawamura (1932). However, attempts to vaccinate rhesus monkeys against *S. japonicum* with adult worm material produced no detectable resistance (Vogel, 1949; Vogel and Minning, 1953).

Lin *et al.* (1954) in a brief report, claimed that a saline suspension of adult *S. japonicum* homogenate gave 34% protection against challenge in mice after eight weekly injections and 56% protection in a second experiment after 14 injections. Very small amounts of highly diluted homogenate were used. In later work (Sadun and Lin, 1959) larger amounts of antigen given intra-

peritoneally in three weekly doses resulted in only 20% resistance against challenge but this low level was statistically significant.

Attempts to vaccinate against *S. mansoni* with dead material have been even less successful. Watts (1949) used saline extracts of adult worms which had been frozen and dried. A large group of mice were vaccinated five times at weekly intervals with highly diluted extract equivalent to about 100 μg of the dried starting material. Following challenge there was a small but statistically significant reduction in the number of adult worms recovered from vaccinated mice compared with controls. Thompson (1954) could not detect any protective effect following vaccination of mice with formalin-killed cercariae although as many as ten injections of a total of 44 000 organisms were given by a variety of routes. Ritchie *et al.* (1962) were unable to vaccinate mice even using homogenates of cercariae, adult worms and eggs in sequence, with the object of exposing the host to the range of antigens derived from these stages during the course of an infection.

A considerable degree of protection was obtained in rats after 21 consecutive daily injections of saline soluble antigens extracted from lyophilized *S. mansoni* (Sadun and Bruce, 1964). However, control rats given the same number of injections of bovine serum albumin showed an equal level of protection, presumably due to non-specific stimulation. Silva and Ferri (1968) used a saline extract of dried *S. mansoni* to vaccinate mice which had been infected four weeks previously. Subcutaneous injections of small amounts of antigen were given twice weekly for four weeks and the number of mice which died were recorded during the following three months. The mortality among control mice was three times greater than in the vaccinated group. No attempt was made to determine whether this method of vaccination had any effect on the numbers of adult worms or caused reduction in the degree of pathology attributable to the formation of granulomata around eggs in the liver. Vaccination of rhesus monkeys with homogenates of cercariae, adult worms or eggs did not stimulate any detectable degree of resistance to challenge (Smithers, 1962). To test the possibility that functional antigens were destroyed during preparation of homogenates Smithers and Terry (1967) injected adult worms, killed by snap-freezing, directly into the portal veins of rhesus monkeys. These dead worms did not induce any resistance to challenge whereas a similar number of live adult schistosomes, introduced directly into the portal veins in the same way, stimulated a high level of immunity.

Murrell *et al.* (1975) have recently reinvestigated the possibility of vaccinating against *S. mansoni* with soluble antigens extracted from adult worms in various ways. Antigens extracted from homogenized worms with buffered saline were concentrated by ultrafiltration and given subcutaneously to mice three times within a week. Freund's complete or incomplete adjuvant, alhydrogel and BCG were tested but no protection against challenge was detected. Soluble antigens obtained by freeze-thawing adult worms instead of homogenization also failed to vaccinate. Antgiens soluble in 3M KCl gave a small amount of protection in one experiment of three. Soluble material obtained by holding large numbers of cercariae in water for several days

at 4°C also gave moderate protection in one experiment but not in two others.

In summary it may be said that very few of the attempts to vaccinate experimental animals with homogenates or extracts of larval or adult *S. mansoni* or *S. japonicum* have given more than marginal protection against challenge. Nevertheless, attempts have been made to increase human resistance to *S. haematobium* by intradermal injections of inactivated cercariae. In the first of these reports (Dodin and Moreau, 1967) schoolchildren in Madagascar, infected with *S. haematobium*, were clinically cured with the drug Niridazole and examined at intervals for reappearance of eggs in the urine, indicating reinfection. Slightly fewer reinfections occurred in a group of children given a single intradermal injection of inactivated *S. mansoni* cercariae, than in a control group. In more extensive tests (Dodin *et al.*, 1969) children clinically cured of *S. mansoni* or *S. haematobium* infections became reinfected within three months but in groups given a single intradermal injection of inactivated homologous cercariae reinfection was delayed for 16–21 months. These results are surprising in view of the general failure to obtain protection in experimental animals with cercarial antigens. Possibly the cercariae, which were inactivated by treatment with a solution containing ascorbic acid and Cu ions, believed to generate free radicals (Dodin and Moreau, 1967) were unusually immunogenic. It would be interesting to know whether cercariae inactivated in this way will vaccinate experimental hosts.

C. VACCINATION WITH SECRETED ANTIGENS

The partial success of vaccination against several nematodes with secreted antigens in the late 1950s might have been expected to encourage a similar approach with schistosomes but surprisingly few attempts to vaccinate with material from cultured organisms have been reported. One of the lines of evidence supporting the view that functional antigens are secreted by nematodes and cestodes has been the stimulation of immunity by living organisms held inside diffusion chambers implanted in the host. This technique has not been exploited with schistosomes, with the exception of one attempt using adult *S. mansoni* in mice, which resulted in stimulation of antibody formation but not protection against challenge (Hillyer *et al.*, 1970).

Levine and Kagan (1960) attempted to vaccinate mice with antigens which had diffused out of large numbers of cercariae or adult worms held overnight in distilled water. Groups of mice injected with antigens released from cercariae, adult worms, or a combination of these materials, survived slightly longer following a lethal challenge infection than a control group. These antigens were described as "metabolic products" but the adult schistosomes would have died rapidly in distilled water and the antigens diffusing out of the dead worms can hardly be regarded as secreted material. Sadun and Lin (1959) obtained antigens more correctly described as "metabolic products" from 50–100 adult *S. japonicum*, maintained for 72 h at 37°C in a protein-free medium, containing serum ultrafiltrate diluted with a balanced salt solution.

The medium containing antigens was lyophilized and given in three intraperitoneal injections at weekly intervals, without adjuvant. A group of 30 mice vaccinated in this way showed only 20% protection against challenge compared with a control group but the difference was statistically significant. Murrell and Clay (1972) collected antigens secreted by adult *S. mansoni* into a protein-free tissue culture medium, previously shown to allow prolonged survival of adult worms (Senft and Senft, 1962). Medium in which 40 schistosomes had been maintained for 36–48 h was concentrated 250 times by ultrafiltration through a cut-off membrane retaining molecules above 10000 M.W. Secreted antigens, in incomplete Freund's adjuvant, were given subcutaneously to mice followed by four intraperitoneal doses of the same material with an alum adjuvant, over a total period of 40 days. Following a challenge with cercariae about 40% fewer adult worms were recovered from vaccinated mice than from a control group given the adjuvants alone.

This very limited information suggests that antigens secreted into culture medium by adult *S. japonicum* and *S. mansoni* are worth further examination as possible functional antigens. So far there have been no reports of vaccination attempts using antigens secreted by schistosomula although these stages develop well in culture (Clegg, 1965).

D. NATURE AND ORIGIN OF ANTIGENS SECRETED BY SCHISTOSOMES

1. *Circulating antigens*

Smithers and Terry (1976) have recently summarized work on the circulating antigen originally detected by Berggren and Weller (1967) in the serum of hamsters heavily infected with *S. mansoni*. The antigen is heat stable, has a molecular weight of less than 10000 and can be readily extracted from adult worms. The same antigen appears to be secreted by *S. haematobium* and *S. japonicum* and has been detected in the urine as well as the serum of various experimental hosts and man. The main interest in circulating antigen has been in the possibility of developing a specific test to determine the presence and intensity of schistosome infections. However, although the antigen is relatively small, it may well be of significance in the induction of immunity and Carlier *et al.* (1975) have presented evidence relating this circulating antigen to the formation of IgE.

2. *Antigens secreted* in vitro

The material secreted by *S. mansoni* into a protein-free chemically defined medium, which was used by Murrell and Clay (1972) to partially vaccinate mice, has been examined by Murrell *et al.* (1974). Analysis on polyacrylamide gel and by immunoelectrophoresis detected fifteen protein components. Only two of these reacted with antibodies in serum from animals infected with *S. mansoni*. The total output of secreted material was quite low, about 0·1–0·2 μg^{-1} worm day^{-1}. Culture of 50–100000 worms for 48 h was necessary to

recover 15–20 mg of protein. However, comparison of secreted antigens with extracts of adult worms by immunodiffusion showed that most of the secreted antigens could be obtained much more readily from whole worms by repeated freezing and thawing or extraction in 3M KCl.

The antigens secreted by adult *S. mansoni* during culture *in vitro* have been analysed by more sensitive radio-labelling techniques (Kusel *et al.*, 1975a). The proteins being actively synthesized by the schistosomes were first labelled by culturing the worms for 24 h in medium containing ^{3}H leucine. During subsequent maintenance in medium without radioactive leucine, labelled proteins appeared progressively in the medium. The labelled proteins were reacted with serum from an immune rhesus monkey and co-precipitated with rabbit antiserum directed against monkey IgG. The precipitates were dissociated in SDS and analysed by electrophoresis on polyacrylamide gel. Seven major antigens were recognized by antibodies in the immune rhesus serum, indicating the high sensitivity of this technique.

Further analysis showed that, of the labelled TCA precipitable material released into the culture medium, which is largely protein, 36% could be removed by co-precipitation using immune rhesus serum (Kusel *et al.*, 1975b). Apparently, most of the proteins released by *S. mansoni* in culture are not detected by antibodies found in infected monkeys. This may indicate that more material is released under culture conditions than in the host or that most of the proteins secreted by adult schistosomes are very poor immunogens.

Absorption of the immune monkey serum with isolated schistosome surface membranes indicated that one third of the antigens recognized by monkey antibodies are derived from the surface membrane. An effective control was performed by similar absorption with mouse liver cell membranes. About half the surface antigens released into the culture medium were in particulate form. This was confirmed by surface labelling of living schistosomes with ^{125}I using the lactoperoxidase method. The majority of proteins in the surface membrane were shown to turn over at about the same rate by a double isotope labelling technique (Kusel and Mackenzie, 1975). However, some soluble proteins in the medium, which shared antigenic determinants with surface antigens, had a much higher turnover rate than any protein in the surface membrane. This evidence may indicate that surface type antigens of schistosomes are released into culture medium by two distinct processes, release of membrane fragments, and a rapid secretory process.

3. *Vaccination with membrane antigens*

The effector mechanism of the immune response stimulated by an infection with *S. mansoni* almost certainly involves recognition by antibody of antigens on the surface membrane of young schistosomula (reviewed by Clegg, 1974; Smithers and Terry, 1976). Most of the surface membrane antigens of the adult schistosomes are known to be present on the surface membrane of schistosomula (Kusel, 1972; Kusel *et al.*, 1975a). Surface membrane proteins of the adult worm are continuously released into culture medium and some of

these represent antigens which stimulate antibody formation in an infected host. Thus surface membrane antigens seem to be good candidates as functional antigens.

Ramalho-Pinto *et al.* (1976) have used the carrier effect to show that helper T-cell activity against antigens in the surface membrane of schistosomula is increased during an infection of mice with *S. mansoni*. If a hapten coupled to a protein carrier is injected into an animal which has been previously immunized against the carrier protein, there is enhanced production of antibody to the hapten, due to co-operation between T-cells sensitized to the carrier and B-cells directed against the hapten (Mitchison, 1971). Formalin-fixed schistosomula were coated with the hapten trinitrophenol (TNP) and injected intravenously into mice infected with *S. mansoni* and normal controls. Assay of spleen cells four days later for antibody production, using the Jerne plaque method, showed that T-cells of infected mice had been sensitized to carrier proteins in the surface of schistosomula. Comparable T-cell priming was induced by intravenous injection of only 30 formalin-killed schistosomula or 10 μg of isolated adult schistosome surface membranes. Vaccination in this way did not, however, induce protective immunity against challenge. Increasing the number of fixed schistosomula from 30 to 1000 stimulated a detectable but very low level of protection. The failure of vaccination with dead schistosomula or isolated adult surface membrane suggests that the antigens are not being presented in the most efficient way (Ramalho-Pinto *et al.*, 1976). An alternative possibility is that antigen from some other site in the parasite plays an essential role in the induction of protective immunity.

E. OTHER APPROACHES TO VACCINATION

1. *Vaccination with target antigens*

One rational approach to vaccination is based on the analysis of the effector mechanism of the immune response against schistosomes. If the target antigen of this mechanism could be isolated it would be the obvious candidate for the functional antigen.

Clegg and Smithers (1972) detected an IgG antibody in the serum of hyper-immune rhesus monkeys which could kill schistosomula of *S. mansoni* during culture *in vitro*. In conjunction with complement this antibody damages the surface membrane of the schistosomula (McLaren *et al.*, 1975). The lethal antibody could be absorbed with surface membranes isolated from adult schistosomes and large amounts of the antigen responsible for absorption were detected in crude, saline insoluble, material from homogenized worms (Sher *et al.*, 1974). Fractionation of the crude insoluble material after solubilization in SDS was carried out by column chromatography. A fraction containing about four proteins was isolated, which absorbed the lethal antibody from immune rhesus serum. Vaccination of rats with this fraction in complete Freund's adjuvant stimulated the formation of high levels of lethal antibody but the rats were not protected against challenge. It was concluded that the lethal antibody detected *in vitro* is not involved in the effector mechanism

operating in the host or, if it is involved, it must act in co-operation with another antibody or sensitized cell. Murrell *et al.* (1975) have similarly shown that lethal antibody can be induced by vaccination of mice with various extracts of adult schistosomes without stimulating protective immunity. These authors suggest that lethal antibody may only be able to act on schistosomula in the skin, in the short period during which they are vulnerable, if local capillary permeability is enhanced by an immediate type hypersensitivity reaction involving mast cells and IgE.

Several other immune mechanisms capable of damaging schistosomula *in vitro* have been described, which involve antibody acting in co-operation with various non-sensitized cells. Neutrophil polymorphs can greatly increase the rate at which schistosomula are killed by an IgG_2 antibody from immune rats acting in conjunction with complement (Dean *et al.*, 1974). Normal rat macrophages can damage schistosomula opsonized with an IgG_1 antibody in the absence of complement (Perez, 1974). Complexes of rat IgE and schistosome antigen can activate macrophages which then bind to, and damage, schistosomula *in vitro* (Capron *et al.*, 1975, 1976). Human IgG antibody of unknown class can cooperate with eosinophils in damage to schistosomula detected by a ^{51}Cr release assay (Butterworth *et al.*, 1975). This mechanism is of particular interest because eosinophils have been implicated in the immune mechanism operating in mice. Following severe depletion of eosinophils in immune mice with a highly specific anti-eosinophil serum, protection against reinfection was substantially reduced (Mahmoud *et al.*, 1975). Isolation of the target antigen of the eosinophil-mediated effector mechanism has not been reported but this is clearly a most promising candidate antigen for vaccination against *S. mansoni.*

2. *Vaccination with the target molecules of drugs*

Bout *et al.* (1976) have used a new and unusual approach to vaccination, which depends on isolation of the schistosome molecules which are the targets for schistosomicidal drugs. Since binding of the drug to the target molecule causes serious damage to the parasite these molecules must be of crucial importance in the physiology of the parasite. Antibody directed against such a target molecule might be expected to have a similarly damaging effect.

Target molecules were purified from extracts of adult *S. mansoni* by affinity chromatography using anti-schistosome drugs as ligands. Alternatively, insoluble drugs were used directly to absorb target molecules, without attachment to a supporting matrix. Rats were vaccinated with target antigens isolated with the drugs Antimony potassium tartrate and Amphotalide. 50 μg of target antigens, mixed with diphtheria toxoid and Freund's complete adjuvant, was injected three times over a 50 day period. Control rats were given the adjuvants alone. Substantial immunity against challenge was observed in vaccinated rats but attempts to vaccinate mice with similar target antigens were not successful. The target antigens purified with Antimony potassium tartrate were shown to include the enzymes glucose-6-phosphate

dehydrogenase, malic dehydrogenase and α-carboxylesterase. These investigations are evidently at an early stage of development but the approach shows considerable promise.

F. NON-SPECIFIC RESISTANCE TO *S. MANSONI*

1. *Inflammatory reactions*

Recent investigations have shown that non-specific inflammation, induced in the pathway followed by migrating schistosomula, can result in efficient destruction of the organisms. Smith *et al.* (1975) induced marked infiltration of neutrophil polymorphs and some macrophages into the lung tissues of hamsters by intravenous injection of large numbers of formalin-killed *E. coli*, three days after infection with *S. mansoni* cercariae. Two days later, recovery of schistosomula from the lungs was depressed by as much as 80% by comparison with recoveries from the lungs of controls. Recovery of adult schistosomes six weeks after infection confirmed that the reduced recoveries from the lungs represented elimination of the schistosomula. Lower levels of destructon of migrating schistosomula were obtained when lung inflammation was induced by intravenous injection of the purified proteins, human serum albumin or spider crab haemocyanin, in soluble form, complexed with specific antibody or heat-aggregated. Non-specific inflammation of the lungs is evidently able to cause destruction of schistosomula, migrating through the lung capillaries, but the mechanism remains obscure. It may well involve damage to schistosomula by lysosomal enzymes of polymorphs or macrophages released during phagocytosis of the dead bacteria, or purified proteins, used to stimulate inflammation.

Following penetration of the skin by cercariae, the resulting schistosomula remain quiescent in the dermis for at least 48 h before migrating to the lungs. Fauve and Dodin (1976) induced inflammation in the skin of mice by subcutaneous injection of a mixture of talc and calcium phosphate, 3 or 17 days before infection with cercariae. In 12 of 15 mice treated in this way very substantial reductions in the level of infection were observed. Thermal inflammation in the tail skin of mice, produced by mild heating at 50°C for 3 min, one hour before exposure of the tail to cercariae has been reported to block infection completely (Gysin and Le Coroller, 1976).

These demonstrations of destruction of schistosomula by localized non-specific inflammatory reactions do not suggest any realistic approach to vaccination. Even if chronic inflammation could be induced in the skin, it would hardly be practicable to involve the large areas of human skin through which invasion by cercariae may occur during wading or washing in infected water. However, inflammatory reactions induced by a variety of agents can kill schistosomula very efficiently and study of the mechanism of these reactions should reveal the ways in which they differ from the mechanism which operates in acquired immunity. It may then be possible to induce a specific immune form of inflammation against schistosomula which is more efficient then the response acquired during an infection.

2. *BCG*

The living attenuated strain of *Mycobacterium bovis,* Bacillus Calmette-Guerin (BCG) has shown considerable activity against *S. mansoni.* Hamsters were given 7·5 mg of BCG by the scarification method on abdominal skin, three weeks before infection with *S. mansoni* cercariae, through the same area of skin (Capron and Lesoin, 1969). BCG treated hamsters showed about 50% protection by comparison with controls given the same scarification without BCG. Fauve and Dodin (1976) completely protected mice against *S. mansoni* by a subcutaneous injection of $1{\cdot}5 \times 10^8$ viable units of BCG, ten days before infection. In a second experiment only seven of ten mice were fully protected. Killed organisms were not effective and living organisms failed to protect when injected in very concentrated form. These conclusions, however, were based on single experiments. Intravenous injection of C57BL inbred mice with 1 mg BCG, 14 days before infection, stimulated complete protection against *S. mansoni* in one experiment and 80% protection in another (Bout *et al.*, 1977). One form of BCG (BCG/F) was effective but another, (BCG/S) intended for administration by scarification, gave no result, although BCG/S had previously been effective in hamsters (Capron and Lesoin, 1969).

The limited information available shows that, at least on some occasions, vaccination with living BCG can give complete protection against infection with *S. mansoni* in mice. So far there is no indication of the mechanism involved but the activity of BCG therapy against animal and human neoplasms is believed to involve non-specific activation of macrophages (review by Laucius *et al.*, 1974).

Capron and Lesoin (1969) reported that scarification of hamsters with BCG, mixed with crude homogenate of adult *S. mansoni,* induced higher levels of protection than administration of BCG alone. In view of the subsequent evidence that BCG alone can protect completely mice on some occasions, much more experimental evidence will be needed before this apparent adjuvant effect of BCG with schistosome antigens can be accepted.

G. VACCINATION WITH HETEROLOGOUS ANTIGENS

Hillyer *et al.* (1975) have attempted to vaccinate mice and hamsters against *S. mansoni* with antigen prepared from *Fasciola hepatica.* A total quantity of 9 mg of crude *Fasciola* homogenate in Freund's complete or incomplete adjuvant was given in three injections per week for three weeks. A partially purified fraction of the crude material, obtained by column chromatography on Sephadex G200, was tested in the same way. The maximum level of protection recorded against challenge with *S. mansoni* was 39% in mice and 27% in hamsters. *Fasciola hepatica* and *S. mansoni* have been shown by immunodiffusion to have at least five common antigens (Capron *et al.*, 1968) and the partially purified fraction of *Fasciola* material contained at least some of these common antigens (Hillyer *et al.*, 1975). These cross-reacting antigens could account for the moderate protective effect against *S. mansoni* following

vaccination with *Fasciola* material. Reyes and Hillyer (1976) have briefly reported that much higher levels of protection against *S. mansoni* (67–72%) can be obtained in mice by vaccination with crude *Fasciola* antigen in combination with the polynucleotide adjuvant, poly AU. However, the adjuvant used alone gave 52% protection. This very interesting non-specific effect of poly AU against *S. mansoni* will, no doubt, encourage much further investigation.

Cox and Oliver-Gonzales (1970) reported that intraperitoneal injections of disrupted membranes from rhesus monkey erythrocytes could completely destroy adult *S. mansoni* in the mesenteric veins of mice. Extracts of guinea-pig kidney (Cox and Oliver-Gonzalez, 1969) and snail haemolymph (Oliver-Gonzalez, 1968) were previously found to have a similar but less decisive effect on adult schistosomes. Dean and Gadd (1973) were completely unable to reproduce these results using disrupted stromata of erythrocytes from rhesus monkeys and several other animals. Similarly Chiriboga *et al.* (1971) could not repeat the effect claimed for snail haemolymph. Both Dean and Gadd (1973) and Chiriboga *et al.* (1971) have suggested that the effects on adult *S. mansoni*, originally observed following intraperitoneal administration of these heterologous antigens, were probably due to bacterial contamination of the preparations. Many species of bacteria have been shown to colonize the intestine of adult *S. mansoni* and destroy the worms, following intravenous or intraperitoneal injection in mice with infections of more than three weeks duration (Ottens and Dickerson, 1969).

H. VACCINATION AGAINST *FASCIOLA HEPATICA*

Kerr and Petrovitch (1935) attempted to vaccinate rabbits with a series of nine intraperitoneal injections of a saline suspension of powdered material prepared from dried, whole adult *Fasciola*. Following a challenge infection with metacercariae marginally fewer adult worms were recovered from vaccinated rabbits than from controls. Urquhart *et al.* (1954) tested soluble antigens extracted from homogenates of adult worms. After precipitation with alum adjuvant, the antigens were given intramuscularly to rabbits three or six times at 8–10 day intervals. Recoveries of challenge infections from groups of vaccinated rabbits were not significantly lower than recovery from a control group but the worms were smaller. Total nitrogen estimations of the worms from the vaccinated and control groups confirmed the difference in size. Healy (1955) and Ross (1967) found no evidence of protection in rabbits after vaccination with extracts of adult or near-adult *Fasciola*. Hughes (1962) using lyophilized adult worms, found a significant level of protection in one experiment with rabbits but in a second, larger, experiment no immunity could be detected. Dragneva (1972) similarly reported failure to vaccinate rabbits with extracts of adult worms or lyophilized material but Gigitashvili and Mdivnishvili (1972) observed small reductions in a challenge infection following vaccination of guinea-pigs with dried, powdered, adult *Fasciola*. Sinclair and Joyner (1974) injected extracts of adult worms directly into the livers of infected rabbits. This procedure caused considerable damage to the

liver tissue but many of the young flukes of a subsequent challenge infection were killed while migrating through the treated livers.

2. *Secreted antigens*

Dragneva (1972) reported briefly that vaccination of rabbits, with saline in which adult worms had been incubated for several hours, gave a significant reduction in a challenge infection. Lang (1976) has now achieved high levels of protection against *Fasciola* in mice, using antigens secreted into a tissue culture medium by immature 16-day old worms. Mice given a single intraperitoneal injection of secreted antigens, collected from 40 immature worms over a 4 h period, showed no protection against a challenge infection. However, when medium containing secreted antigens was harvested at intervals of 2, 7, 12 and 24 hours and immediately injected into mice, a high level of immunity was induced. Following a small challenge infection 30 days after vaccination no worms developed in 75% of a group of 20 treated mice and the number of adult worms recovered from the remaining mice was 83% below the mean recovery from a control group. Fresh homogenates of immature worms failed to vaccinate. Unfortunately these results were taken from a single experiment but further work with this system could prove very interesting.

I. CONCLUSIONS AND COMMENTS

Attempts to vaccinate against schistosomiasis with dead antigenic material have been almost completely unsuccessful up to the present time. High levels of protection have been observed following non-specific stimulation of mice with BCG but the effect has been very variable and much more information will be needed before the potential value of this approach can be assessed. The synthetic polynucleotide, Poly AU, has also been reported to stimulate a considerable degree of non-specific protection in mice against challenge with *S. mansoni* (Reyes and Hillyer, 1976). Protection by BCG may be due to the known non-specific effect of this agent on macrophages (see Laucius *et al.*, 1974) and double-stranded polynucleotides are also capable of activating macrophages non-specificially (Alexander and Evans, 1971). However, both BCG and synthetic polynucleotides have a variety of stimulatory effects on other cells involved in the immune response (reviews by Laucius *et al.*, 1974; Johnson, 1973) and, at present, there is no direct evidence on their mechanism of action against schistosomes.

Dead vaccines have also given very poor results with *Fasciola hepatica* but the recent report by Lang (1976) of a high level of protection in mice, following administration of antigens secreted into culture medium, is encouraging. There have been very few trials of secreted antigens, collected from culture medium, in vaccination against schistosomes. In view of the fact that secreted antigens have been identified as the functional antigens responsible for induction of protective immune responses in many nematodes and larval cestodes, antigens secreted by schistosomes clearly require much deeper

investigation. Surface membrane proteins are known to be released by *S. mansoni* during culture *in vitro* and considerable attention has been given to antigens from this site because there is good evidence that the immune effector mechanism acts against target antigens at the surface. So far, attempts to vaccinate with surface membrane antigens have not been successful. In fact, only about one third of the proteins secreted by schistosomes in culture are derived from the surface membrane (Kusel *et al.*, 1975b) and antigens originating from other sites in the parasite may well be involved in stimulation of a protective immune response.

Failure to stimulate more than marginal levels of protection against schistosomes with dead vaccines may not be due to absence of functional antigens from the preparations which have been tested. It is quite possible that schistosome functional antigens have unusually low immunogenicity. The structural properties of antigens which make them effective immunogens are very poorly understood at present but the determinant sites (epitopes) are not the only significant parts of the molecule. Some unknown structural features, in addition to the epitopes, affect the efficiency with which antibody formation is stimulated and these have been collectively described as the adjuvanticity of the antigen (WHO, 1976b). Poor immunogens may be made more effective by covalent coupling to molecules with high adjuvanticity, such as tetanus toxoid. The immune response to poor immunogens can often, of course, be greatly stimulated by use of one or other of the commonly used adjuvants which are simply administered with the antigen. A very useful survey of the adjuvants currently in use or being developed, with discussion of possible mechanisms of action, is available (WHO, 1976b).

Live vaccines against schistosomes using irradiated cercariae or schistosomula are also being investigated (reviewed by Smithers, 1976). The very short survival period of the infective cercarial stage is a serious problem but this may be overcome by cryopreservation of the irradiated organisms. High levels of protection in experimental hosts have only been obtained by administering large numbers of irradiated cercariae over a considerable period. This procedure is not considered likely to lead to development of a satisfactory method for human vaccination (Smithers, 1976) but it may be suitable for use against *S. mattheei* in sheep (Taylor *et al.*, 1976).

Whether a dead or living vaccine is eventually developed against schistosomes, supply of sufficient antigen for protection of the very large numbers of people at risk is likely to present considerable difficulty. As with nematodes and cestodes, continuous cultivation of the parasite *in vitro* is a highly desirable objective. Encouraging progress has been made with culture of the larval stages, which develop in the snail intermediate host, although production of cercariae has not yet been achieved (Hansen, 1975; Basch and DiConza, 1977). Development from the cercariae to adult worm occurs under simple culture conditions but egg formation does not take place *in vitro* (Clegg, 1965). An alternative to continuous culture, which has been vigorously advocated by Weller (1976) involves cultivation of cell lines isolated from schistosomes. Maintenance of schistosome cells *in vitro* has been achieved after considerable effort but the cells do not multiply (Weller, 1976). Mass cultivation of a

suitable cell line synthesizing the functional antigen might be the ideal method for collection of large quantities of schistosome antigen for vaccine production. This approach could be equally applicable to nematodes and cestodes and some preliminary work has been done with nematodes (see Silverman and Hansen, 1971) and with cells derived from the germinal layer of *Echinococcus multilocularis* (see Smyth, 1969).

Although progress towards dead vaccines against trematodes has been disappointingly slow, the many examples of successful vaccination against nematodes and larval cestodes may be considered sufficient cause for guarded optimism that the forecasts for development of experimental vaccination against schistosomiasis by the early 1980s (Hoffman, 1975) may be realized.

References

Alexander, P. and Evans, R. (1971). Endotoxin and double stranded RNA render macrophages cytotoxic. *Nature New Biology* **232**, 76–78.

Bachman, G. W. and Molina, R. (1933). Resistance to infestation with *Trichinella spiralis* in hogs. *American Journal of Hygiene* **18**, 266–294.

Basch, P. F. and DiConza, J. J. (1977). *In vitro* development of *Schistosoma mansoni* cercariae. *Journal of Parisitology* **63**, 245–249.

Berggren, W. L. and Weller, T. H. (1967). Immunoelectrophoretic demonstration of specific circulating antigen in animals infected with *Schistosoma mansoni*. *American Journal of Tropical Medicine and Hygiene* **16**, 606–612.

Berntzen, A. K. (1965). Comparative growth and development of *Trichinella spiralis in vitro* and *in vivo*, with a re-description of the life cycle. *Experimental Parasitology* **16**, 74–106.

Berntzen, A. K. (1974). Effects of environment on the growth and development of *Trichinella spiralis in vitro*. *In* "Trichinellosis, Proceedings of the Third International Congress on Trichinellosis" (C. W. Kim, ed.), pp. 25–30. Intext Educational, New York.

Bout, D., Dupas, H., Capron, M., Tran Van Ky, P. and Capron, A. (1976). Drugs as ligands of immunogenic molecules in parasites: an approach to the isolation of target-antigens. *In* "Biochemistry of Parasites and Host-Parasite Relationships" (Van den Bossche, ed.), pp. 325–333. Elsevier, North-Holland, Amsterdam.

Bout, D., Dupas, H., Carlier, Y., Afchain, D. and Capron, A. (1977). Protection of mice against *Schistosoma mansoni* with BCG. *Medical Science: Immunology and Allergy; Microbiology, Parasitology and Infectious Diseases; Pharmacology* **5**, 47.

Bruce, R. G. (1974). The formation and utilization of stichosome secretory globules in *Trichinella spiralis*. *In* "Trichinellosis, Proceedings of the Third International Congress on Trichinellosis" (C. W. Kim, ed.), pp. 49–57. Intext Educational, New York.

Buecher, E. J. and Hansen, E. L. (1971). Mass cultivation of *Caenorhabditis elegans*. *Journal of Nematology* **3**, 199.

Butterworth, A. E., Sturrock, R. F., Houba, V., Mahmoud, A. A. F., Sher, A. and Rees, P. H. (1975). Eosinophils as mediators of antibody-dependent damage to schistosomula. *Nature London* **256**, 727–729.

Campbell, D. H. (1936). Active immunization of Albino rats with protein fractions from *Taenia taeniaeformis* and its larval form *Cysticercus fasciolaris*. *American Journal of Hygiene* **23**, 104–113.

Campbell, C. H. (1955). The antigenic role of the excretions and secretions of *Trichinella spiralis* in the production of immunity in mice. *Journal of Parasitology* **41**, 483–491.

Capron, A. and Lesoin, M. A. (1969). Rôle protecteur de BCG dans la schistosomiase expérimentale a *S. mansoni*. *Comptes rendus Academie de Sciences (Paris)* **269**, 2110–2112.

Capron, A., Biguet, J., Vernes, A. and Afchain, D. (1968). Structure antigènique des helminthes. Aspects immunologiques des rélations hôte–parasite. *Pathologie et Biologie* **16**, 121–138.

Capron, A., Dessaint, J. P., Capron, M. and Bazin, H. (1975). Specific IgE antibodies in immune adherence of normal macrophages to *Schistosoma mansoni* schistosomules. *Nature, London* **253**, 474–475.

Capron, A., Dessaint, J. P., Camus, D. and Capron, M. (1976). Some immune mechanisms in host-parasite relationship. *In* "Biochemistry of Parasites and Host-Parasite Relationships" (Van den Bossche, ed.) pp. 263–282. Elsevier, North-Holland, Amsterdam.

Carlier, Y., Bout, D., Bina, J. C., Camus, D., Figueiredo, J. F. M. and Capron, A. (1975). Immunological studies in human schistosomiasis. I. Parasitic antigen in urine. *American Journal of Tropical Medicine and Hygiene* **24**, 949–954.

Cavier, R. and Hawking, F. (1973). "Chemotherapy of Helminthiasis, volume I, International Encyclopaedia of Pharmacology and Therapeutics, Section 64, Pergamon Press.

Chandler, A. C. (1932). Susceptibility and resistance to helminthic infections. *Journal of Parasitology* **18**, 135–152.

Chandler, A. C. (1936). Studies on the nature of immunity to intestinal helminths. III. Renewal of growth and egg production in *Nippostrongylus* after transfer from immune to non-immune rats. *American Journal of Hygiene* **23**, 46–54.

Chipman, P. B. (1957). The antigenic role of the excretions and secretions of adult *Trichinella spiralis* in the production of immunity in mice. *Journal of Parasitology* **43**, 593–598.

Chiriboga, J., Ritchie, L. S., Oliver-Gonzalez, J., Brown, R. and Lopez, V. A. (1971). Bacteria in the curative effect of hemolymph of *Biomphalaria glabrata* on *Schistosoma mansoni*-infected mice. *American Journal of Tropical Medicine and Hygiene* **20**, 672–678.

Chute, R. M. (1956). The dual antibody response to experimental *trichiniasis*. *Proceedings of the Helminthology Society of Washington* **23**, 49–58.

CIBA Foundation Symposium 25 (new series) (1974). "Parasites in the immunized host: mechanisms of survival". Elsevier, Excerpta Medica, North-Holland, Amsterdam.

Clegg, J. A. (1965). *In vitro* cultivation of *Schistosoma mansoni*. *Experimental Parasitology* **16**, 133–147.

Clegg, J. A. (1974). Host antigens and the immune response in schistosomiasis. *In* "Parasites in the Immunized Host: Mechanisms of Survival" (CIBA Foundation Symposium 25: new series) pp. 161–183. Elsevier, Excerpta Medica, North-Holland, Amsterdam.

Clegg, J. A. and Smithers, S. R. (1972). The effects of immune rhesus monkey serum on schistosomula of *Schistosoma mansoni* during cultivation *in vitro*. *International Journal for Parasitology* **2**, 79–98.

Clegg, J. A. and Smyth, J. D. (1968). Growth, development and culture methods: parasitic platyhelminths. *In "Chemical Zoology"* volume II (M. Florkin and B. T. Scheer, eds) pp. 395–446. Academic Press, New York and London.

Cox, K. B. and Oliver- Gonzalez, J. (1969). Effects of guinea-pig kidney extract

positive for Forssman antigen on adult *Schistosoma mansoni* in mice. *American Journal of Tropical Medicine and Hygiene* **18**, 683–687.

Cox, K. B. and Oliver-Gonzalez, J. (1970). The effects of rhesus-monkey erythrocyte stromata and hemolysate in experimental murine schistosomiasis mansoni. *American Journal of Tropical Medicine and Hygiene* **19**, 284–288.

Crandall, C. A. and Arean, V. M. (1965). The protective effect of viable and non-viable *Ascaris suum* larvae and egg preparations in mice. *American Journal of Tropical Medicine and Hygiene* **14**, 765–769.

Dean, D. A. and Gadd, P. (1973). Treatment of *Schistosoma mansoni* infection with rhesus monkey stromata: two unsuccessful attempts. *International Journal for Parasitology* **3**, 661–663.

Dean, D. A., Wistar, R. and Murrell, K. D. (1974). Combined *in vitro* effects of rat antibody and neutrophilic leukocytes on schistosomula of *Schistosoma mansoni*. *American Journal of Tropical Medicine and Hygiene* **23**, 420–428.

Denham, D. A. and McGreevy, P. B. (1977). Brugian filariasis: epidemiological and experimental studies. *In* "Advances in Parasitology." Volume 15 (B. Dawes, ed.) pp. 243–309. Academic Press, New York and London.

De Rosa, F., Dottorini, S., Stagni, G. R. and Paulizzi, S. (1974). Immunogenic fractions of *Echinoccoccus granulosus* – A preliminary report. In "Proceedings of the Third International Congress of Parasitology," Volume I, pp. 561–562. Facta, Vienna.

Despommier, D. D. and Müller, M. (1970a). Functional antigens of *Trichinella spiralis*. *Journal of Parasitology* **56**, 76.

Despommier, D. D. and Müller, M. (1970b). The stichosome of *Trichinella spiralis:* its structure and function. *Journal of Parasitoloty* **56**, 76–77.

Despommier, D. D. and Müller, M. (1976). The stichosome and its secretion granules in the mature muscle larvae of *Trichinella spiralis*. *Journal of Parasitology* **62**, 775–785.

Despommier, D. D. and Wostmann, B. S. (1968). Diffusion chambers for inducing immunity to *Trichinella spiralis* in mice. *Experimental Parasitology* **23**, 228–233.

Despommier, D. D., Müller, M., Jenks, B. and Fruitstone, M. (1974). Immunodiagnosis of human trichinosis using counter-electrophoresis and agar gel diffusion techniques. *American Journal of Tropical Medicine and Hygiene,* **23** 41–44.

Despommier, D. D., Campbell, W. C. and Blair, L. S. (1977). The *in vivo* and *in vitro* analysis of immunity to *Trichinella spiralis* in mice and rats. *Parasitology* **74**, 109–119.

Dodin, A. and Moreau, J. P. (1967). Résultat des essais de protection de l'homme contre la réinfestation bilharzienne au moyen de cercaires inactivées. *Comptes Rendus Academie de Sciences (Paris)* 264, 1538–1540.

Dodin, A., Moreau, J. P. and Ramahandry, G. (1969). Résultats des essais de protection contre la réinfestation bilharzienne (*S. haematobium* et *S. mansoni*) après traitement en zone d'endémie au moyen de cercaires inactivées. *In* "Santé et Développement Volume I" OCAM, Tananarive, Madagascar.

Dragneva, N. (1972). Vaccination against *Fasciola hepatica* with somatic and metabolic antigens. *Izvestiya na Tsentralnata Khelmintlogichna Laboratoriya* **15**, 81–87.

Edwards, A. J., Burt, J. S. and Ogilvie, B. M. (1971). The effect of immunity upon some enzymes of the parasitic nematode *Nippostrongylus brasiliensis*. *Parasitology* **62**, 339–347.

Ewart, A. and Olson, L. J. (1961). The use of a mouse oral LD50 to evaluate the

immunogenicity of *Trichinella* metabolic antigens. *Texas Report in Biology and Medicine* **12**, 580–584.

Fauve, R. M. and Dodin, A. (1976). Influence d'une réaction inflammatoire provoquée par le BCG un par un irritant non biodégradable sur la résistance des souris à la bilharziose. *Comptes Rendus Academie de Sciences (Paris)* **282**, 131–134.

Feng, L. C. (1937). Attempts to immunize dogs against infection with *Dirofilaria immitis* Leidy, 1856. *Festschrift Nocht* (1937) *Herausgegeben vom Institüt für Schiffs- und Tropenkrankheiten, Hamburg*, pp. 140–142.

Ferguson, D. L., Rhodes, M. B., Marsh, C. L. and Payne, L. C. (1969). Resistance of immunized animals to infection by the larvae of the large roundworm of swine (*Ascaris suum*). *Federation Proceedings* **28**, 497

Forsek, Z. and Rukavina, J. (1959). Eksperimentalna imunizacija pasa protiv *Echinococcus granulosus*. I. Prva zapazanja. *Veterinaria (Sarajevo)* 8, 479–482.

Gallie, G. S. and Sewell, M. M. H. (1976). Experimental immunisation of six-month old calves against infection with the cysticercus stage of *Taenia saginata*. *Tropical Animal Health Production* **8**, 233–242.

Gemmell, M. A. (1962a). Species specificity of the immunogenic complexes of the tapeworm hexacanth embryo. *Nature, London* **194**, 701–702.

Gemmell, M. A. (1962b). Natural and acquired immunity factors interfering with development during the rapid growth phase of *Echinococcus granulosus* in dogs. *Immunology* **5**, 496–503.

Gemmell, M. A. (1964a). Tapeworm infections. I, Species specificity of hexacanth embryos in protecting sheep against *Taenia hydatigena*. *Immunology* **7**, 489–499.

Gemmell, M. A. (1964b). Species specificity of the immunogenic complexes of the tapeworm hexacanth embryo. *Nature, London* **204**, 705–707.

Gemmell, M. A. (1965a). Immunological responses of the mammalian host against tapeworm infections. II. Species specificity of hexacanth embryos in protecting rabbits against *Taenia pisiformis*. *Immunology* **8**, 270–280.

Gemmell, M. A. (1965b). Immunological responses of the mammalian host against tapeworm infections. III. Species specificity of hexacanth embryos in protecting sheep against *Taenia ovis*. *Immunology* **8**, 281–290.

Gemmell, M. A. (1966). Immunological responses of the mammalian host against tapeworm infections. IV. Species specificity of hexacanth embryos in protecting against *Echinococcus granulosus*. *Immunology* **11**, 325–336.

Gemmell, M. A. (1976). Immunology and regulation of the cestode zoonoses. *In* "Immunology of Parasitic Infections" (S. Cohen and E. Sadun, eds) pp. 333–358. Blackwell Scientific Publications, Oxford.

Gemmell, M. A. and MacNamara, F. N. (1972). Immune response to tissue parasites. II. Cestodes. *In* "Immunity to Animal Parasites" (E. J. L. Soulsby, ed.) pp. 235–272. Academic Press, New York and London.

Gemmell, M. A., Blundell, S. K. and Macnamara, F. N. (1968). Immunological responses of the mammalian host against tapeworm infections. V. The development of artificially induced immunity to *Taenia hydatigena* in young lambs. *Proceedings of the University of Otago Medical School* **46**, 4–5.

Gemmell, M. A., Blundell, S. K. and Macnamara, F. N. (1969). Immunological responses of the mammalian host against tapeworm infections. IX. The transfer via the colostrum of immunity to *Taenia hydatigena*. *Experimental Parasitology* **26**, 52–57.

Gigitashvili, M. S. and Mdivnishvili, D. A. (1972). Active immunization in experimental fascioliasis. (In Russian) *Sbornik Trudov Nauchno-Issledovatel' Skogo Instituta Meditsinskoi Parazitologci i Tropicheskoi Meditsiny* **16**, 35–37.

Gross, J. and Lapiere, C. M. (1962). Collagenolytic activity in amphibian tissues: a tissue culture assay. *Proceedings of the National Academy of Sciences*, U.S.A **48**, 1014–1022.

Guerrero, J. and Silverman, P. H. (1969). *Ascaris suum:* immune reactions in mice. I. La-val metabolic and somatic antigens. *Experimental Parasitology* **26**, 272–281.

Guerrero, J. and Silverman, P. H. (1971). *Ascaris suum:* immune reactions in mice. II. Metabolic and somatic antigens derived from *in vitro* cultures. *Experimental Parasitology* **29**, 110–115.

Gysin, J. and Le Corroller, Y. (1976). The effect of inflammatory reaction of the mouse on experimental infection with *Schistosoma mansoni. Journal of Parasitology* **62**, 697.

Hansen, E. L. (1975). Secondary sporocysts of *Schistosoma mansoni*; the occurrence and cultivation. *Annals of the New York Academy of Science* **266**, 426–436.

Healy, G. R. (1955). Studies on immunity to *Fasciola hepatica* in rabbits. *Journal of Parasitology* **41**, (Supplement) 25.

Heath, D. D. (1973). Resistance to *Taenia pisiformis* larvae in rabbits. I. Examination of the antigenically protective phase of larval development. *International Journal for Parasitology* **3**, 485–489.

Heath, D. D. (1976). Resistance to *Taenia pisiformis* larvae in rabbits: immunization against infection using non-living antigens from *in vitro* culture. *International Journal for Parasitology* **6**, 19–24.

Heath, D. D. and Smyth, J. D. (1970). *In vitro* cultivation of *Echinoccoccus granulosus, Taenia hydatigena, T. ovis, T. pisiformis* and *T. serialis* from oncosphere to cystic larva. *Parasitology* **61**, 329–343.

Hepler, D. I., Luerker, D. C. and Rubin, R. (1976). The efficacy of oral immunization with *Nematospiroides dubius*/mouse model. *Journal of Parasitology* **62**, 491–492.

Herd, R. P. (1977). Resistance of dogs to *Echinococcus granulosus. International Journal for Parasitology* **7**, 135–138.

Herd, R. P., Chappel, R. J. and Biddell, D. (1975). Immunization of dogs against *Echinococcus granulosus* using worm secretory antigens. *International Journal for Parasitology* **5**, 395–399.

Hillyer, G. V., Chiriboga, J., Menendez-Corrada, R., Pellegrino, J. and Liard, F. (1970). An attempt to induce resistance in mice to *Schistosoma mansoni* infection using millipore diffusion chambers. *Revista Instituto Medicina Tropical de Sao Paulo* **12**, 149–151.

Hillyer, G. V., de Diaz, A. del Luno and Garcia-Blanco, M. (1975). A new approach to the study of immunity to schistosomes. *Bolétin de la Association Médica de Puerto Rico* **67**, 381–385.

Hoffman, D. B. (1975). Vaccine development. *In* "Schistosomiasis research—the strategic plan" (D. B. Hoffman, ed.) pp. 10–11. The Ednna McConnell Clark Foundation, New York.

Hogarth-Scott, R. S., Watt, B. J., Ogilvie, B. M. and Rothwell, T. L. W. (1973). The molecular size of nematode acetylcholinesterases and their separation from nematode allergens. *International Journal for Parasitology* **3**, 735–741.

Hughes, D. L. (1962). Observations on the immunology of *Fasciola hepatica* infections in mice and rabbits. *Parasitology* **52**, 4(P).

Jakstys, B. P. and Silverman, P. H. (1969). Effects of heterologous antibody on *Haemonchus contortus* development *in vitro. Journal of Parasitology* **55**, 486–492.

Jarrett, E. E. E. and Urquhart, G. M. (1971). The immune response to nematode infections. *In* "International Review of Tropical Medicine," volume 4 (A. W. Woodruff and D. R. Lincicombe, eds). Academic Press, New York and London.

Jarret, W. F. H., Jennings, F. W., McIntyre, W. I. M., Mulligan, W. and Urquhart G. M. (1960). Immunological studies on *Dictyocaulus viviparus* infection. Immunity produced by the administration of irradiated larvae. *Immunology* **3**, 145–151.

Jenkins, S. N. and Wakelin, D. (1977). The source and nature of some functional antigens of *Trichuris muris*. *Parasitology* **74**, 153–161.

Johnson, A. G. (1973). The adjuvant action of polynucleotides. *Journal of the Reticuloendothelial Society* **14**, 441–448.

Jones, V. E. and Ogilvie, B. M. (1972). Protective immunity to *Nippostrongylus brasiliensis* in the rat. III. Modulation of worm acetylcholinesterase by antibodies. *Immunology* **22**, 119–129.

Kawamura, R. (1932). The recent researches on schistosomiasis in Japan. *In* "Comptes Rendus. Congrès Intérnational de Médicine Tropicale et l'Hygiene (Le Caire)" volume 4, pp. 311–319.

Kerr, K. B. (1935). Immunity against a cestode parasite—*Cysticercus pisiformis*. *American Journal of Hygiene* **22**, 169–182.

Kerr, K. B. (1938). Attempts to induce an artificial immunity against the dog hookworm, *A. caninum* and the pig *Ascaris, A. lumbricoides suum*. *American Journal of Hygiene* **27**, 52–59.

Kerr, K. B. and Petkovitch, O. L. (1935). Active immunity in rabbits to the liver fluke, *Fasciola hepatica*. *Journal of Parasitology* **21**, 319–320.

Kowalski, J. C. and Thorson, R. E. (1972). Immunization of laboratory mice against *Tetrathyridia* of *Mesocestoides corti* (cestoda) using a secretory and excretory antigen and a soluble somatic antigen. *Journal of Parasitology* **48**, 732–734.

Kozar, M. (1969). Influence of the hypersensitivity states in mice on the course of trichinellosis (In Polish). *Wiadomsci Parazytologiczne* **15**, 601–604.

Kusel, J. R. (1972). Protein composition and protein synthesis in the surface membranes of *Schistosoma mansoni*. *Parasitology* **65**, 55–69.

Kusel, J. R. and Mackenzie, P. E. (1975). The measurement of the relative turnover rates of proteins of the surface membranes and other fractions of *Schistosoma mansoni* in culture. *Parasitology* **71**, 261–273.

Kusel, J. R., Sher, F. A., Perez, H., Clegg, J. A. and Smithers, S. R. (1975a). The use of radioactive isotopes in the study of specific schistosome membrane antigens. *In* "Nuclear Techniques in Helminthology Research", pp. 127–143. International Atomic Energy Agency, Vienna.

Kusel, J. R., Mackenzie, P. E. and McLaren, D. J. (1975b). The release of membrane antigens into culture by adult *Schistosoma mansoni*. *Parasitology* **71**, 247–259.

Kwa, B. H. and Liew, F. Y. (1977). Immunity in *taeniasis-cysticercosis*. I. Vaccination against *Taenia taeniaeformis* in rats using purified antigen. *Journal of Experimental Medicine* **146**, 118–131.

Lang, B. Z. (1976). Host-parasite relationships of *Fasciola hepatica* in the white mouse. VII. Effects of anti-worm incubate sera on transferred worms and successful vaccination with a crude incubate antigen. *Journal of Parasitology* **62**, 232–236.

Larsh, J. E. (1961). Experimental *Trichiniasis*. *In* "Advances in Parasitology," volume **1** (B. Dawes, ed.), pp. 213–286. Academic Press, New York and London.

Laucius, J. F., Bodurtha, A. J., Mastrangelo, M. J. and Creech, R. H. (1974).

Bacillus-Calmette-Guérin in the treatment of neoplastic disease. *Journal of the Reticuloendothelial Society* **16**, 347–373.

Lee, D. L. (1970). The fine structure of the excretory system in adult *Nippostrongylus brasiliensis* (Nematoda) and a suggested function for the excretory glands. *Tissue and Cell* **2**, 225–231.

Leland, S. E. (1970). *In vitro* cultivation of nematode parasites. *In* "Advances in Veterinary Science and Comparative Medicine," volume 14 (C. A. Brandly and C. C. Cornelius, eds.) pp. 29–59. Academic Press, New York and London.

Levine, D. M. and Kagan, I. G. (1960). Studies on the immunology of schistosomiasis by vaccination and passive transfer. *Journal of Parasitology* **46**, 787–792.

Lewert, R. M. (1970). Schistosomes. *In* "Immunity to Parasitic Animals," Volume **2** (G. J. Jackson, R. Herman and I. Singer, eds) pp. 981–1008. Appleton-Century-Crofts, New York.

Lin, S., Ritchie, L. S. and Hunter, G. W. (1954). Acquired immunologic resistance against *Schistosoma japonicum. Journal of Parasitology* **40**, 42.

Lindahl, I. L., Kates, K. C., Turner, J. H., Enzie, F. D. and Whitmore, G. E. (1963). Effect of management systems on the growth of lambs and development of internal parasitism. I. Dry lot vs. Two pasture rotation systems. *Journal of Parasitology* **49**, 209–217.

Mahmoud, A. A. F., Warren, K. S., and Peters, P. A. (1975). A role for the eosinophil in acquired resistance to *Schistosoma mansoni* infection as determined by anti-eosinophil serum. *Journal of Experimental Medicine* **142**, 805–813.

Matov, K. and Vasilev, I. D. (1955). Active immunization of dogs against intestinal *Echinococcus* (In Bulgarian). *Izvestiya na Tsentralnata Kheomintologichna Laboratoriya* (Sofia) **1**, 111–125.

McCoy, O. R. (1935). Artificial immunization of rats against *Trichinella spiralis. American Journal of Hygiene* **21**, 200–213.

McLaren, D. J., Clegg, J. A. and Smithers, S. R. (1975). Acquisition of host antigens by young *Schistosoma mansoni* in mice: correlation with failure to bind antibody *in vitro. Parasitology* **70**, 67–75.

Miller, H. M. (1931a). Immunity of the white rat to super infestation with *Cysticerus fasciolaris. Proceedings of the Society for Experimental Biology* **28**, 467–468.

Miller, H. M. (1931b). Further experiments on artificial immunity to a larval cestode. *Proceedings of the Society for Experimental Biology* **28**, 884–885.

Miller, H. M. (1932). Further studies on immunity to a metazoan parasite *C. fasciolaris. Journal of Preventative Medicine* (Baltimore) **6**, 37–46.

Miller, H. M. and Kerr, K. B. (1932). Attempts to immunize rabbits against a larval cestode *Cysticercus pisiformis. Proceedings of the Society for Experimental Biology and Medicine* **29**, 670–671.

Miller, T. A. (1971). Vaccination against the canine bookworm disease. *In* "Advances in Parasitology," volume 9 (B. Dawes, ed.) pp. 153–183, Academic Press, New York and London.

Miller, T. A. (1975). The possibilities for application of the canine hookworm vaccine technology to the prevention and control of hookworm infection and disease in man. *In* "Nuclear Techniques in Helminthology Research" pp. 61–68. International Atomic Energy Agency, Vienna.

Miller, T. A. (1978). *In* "Advances in Parasitology" (W. H. R. Lumsden, J R. Baker and R. L. Muller eds) Volume 16, pp. 333–342, Academic Press, New York and London.

Mills, C. K. and Kent, N. H. (1965). Excretions and secretions of *Trichinella*

spiralis and their role in immunity. *Experimental Parasitology*, **16**, 300–310.

Mishra, N. K. and Marsh, C. L. (1973). *Ascaris suum:* aldolase. I. Purification and immunologic study. *Experimental Parasitology* **33**, 89–94.

Mitchison, N. A. (1971). The carrier effect in the secondary response to hapten protein conjugates. II. Cellular cooperation. *European Journal of Immunology* **1**, 18–27.

Mulligan, W. (1975). Preparation of radiation-attenuated vaccines against helminthic infections. *In* "Nuclear Techniques in Helminthology Research", pp. 1–11. International Atomic Energy Agency, Vienna.

Murata, H. (1939). Immunologische Studien über *Dirofilaria immitis. Fukuoka Acta Medica* **32**, 945–972.

Murray, M. (1972). Immediate hypersensitivity effector mechanisms II. *in vivo* reactions. *In* "Immunity to Animal Parasites" (E. J. L. Soulsby, ed.) pp. 155–190. Academic Press, New York and London.

Murrell, K. D. and Clay, B. (1972). *In vitro* detection of cytotoxic antibodies to *Schistosoma mansoni* schistosomules. *American Journal of Tropical Medicine and Hygiene* **21**, 569–577.

Murrell, K. D., Vannier, W. and Ahmed, A. (1974). *Schistosoma mansoni:* antigenic heterogeneity of excretions and secretions. *Experimental Parasitology* **36**, 316–330.

Murrell, K. D., Dean, D. A. and Stafford, E. E. (1975). Resistance to infection with *Schistosoma mansoni* after immunization with worm extracts or live cercariae: role of cytotoxic antibody in mice and guinea-pigs. *American Journal of Tropical Medicine and Hygiene* **24**, 955–962.

Neilson, J. T. M. (1969). Gel-filtration and disc-electrophoresis of a somatic extract and excretions and secretions of *Haemonchus contortus* larvae. *Experimental Parasitology* **25**, 131–141.

Neilson, J. T. M. (1975). Failure to vaccinate lambs against *Haemonchus contortus* with functional metabolic antigens identified by immunoelectrophoresis. *International Journal for Parasitology* **5**, 427–430.

Ogilvie, B. M. and Jones, V. E. (1971). *Nippostrongylus brasiliensis:* a review of immunity and the host-parasite relationship in the rat. *Experimental Parasitology* **29**, 138–177.

Ogilvie, B. M. and Love, R. J. (1974). Co-operation between antibodies and cells in immunity to a nematode parasite. *Transplantation Reviews* **19**, 147–169.

Ogilvie, B. M. and Worms, M. J. (1976). Immunity to nematode parasites of man with special reference to *Ascaris* hookworms and filariae. *In* "Immunology of Parasitic Infections" (S. Cohen and E. Sadun, eds) pp. 380–407. Blackwell Scientific, Oxford.

Ogilvie, B. M., Rothwell, T. L. W., Bremner, K. C., Schnitzerling, H. J., Nolan, J. and Keith, R. H. (1973). Acetylcholinesterase secretion by parasitic nematodes. I. Evidence for secretion of the enzyme in a number of nematode species. *International Journal for Parasitology* **3**, 589–597.

Ogilvie, B. M., McLaren, D. J. and Worms, M. J. (1975). Studies with *Necator americanus* maintained in hamsters. *In* "Nuclear Techniques in Helminthology Research" pp. 55–60. International Atomic Energy Agency, Vienna.

Oliver-Gonzalez, J. (1968). Effect of hemolymph from *Biomphalaria glabrata* on *Schistosoma mansoni* infection in mice. *Proceedings of the Society for Experimental Biology and Medicine* **128**, 1029–1033.

Ottens, H. and Dickerson, G. (1969). Bacterial invasion of schistosomes. *Nature, London* **223**, 506–507.

Ozawa, M. (1930). Experimental study on acquired immunity to schistosomiasis japonica. *Japanese Journal of Experimental Medicine* **8**, 79–84.

Ozerol, N. H. and Silverman, P. H. (1969). Partial characterization of *Haemonchus contortus* exsheathing fluid. *Journal of Parasitology* **55**, 79–87.

Ozerol, N. H. and Silverman, P. H. (1970). Further characterization of active metabolites from histotropic larvae of *Haemonchus contortus* cultured *in vitro*. *Journal of Parasitology* **56**, 1199–1205.

Perez, H. (1974). "Investigation on the mechanism of protective immunity to *Schistosoma mansoni* in the rat". Ph.D. Thesis, Brunel University, Uxbridge, England.

Poulain, J., Petit, A., Pery, P. and Luffau, G. (1976). Protection des rats contre '*Nippostrongylus brasiliensis*' a l'aide d'antigenes de vers adminstrés par voie orale. *Annales d'Immunologie* (Institut Pasteur) **127C**, 103.

Ramalho-Pinto, F. J., Goldring, O. L., Playfair, J. H. L. and Smithers, S. R. (1976). T cell response to surface components of *Schistosoma mansoni*, *In* "Biochemistry of Parasites and Host-Parasite Relationships" (Van den Bossche, ed.) pp. 291–298. Elsevier, North-Holland, Amsterdam.

Rau, M. E. and Tanner, C. E. (1975). BCG suppresses growth and metastasis of hydatid infections. *Nature, London* **256**, 318–319.

Reyes, C. N. and Hillyer, G. V. (1976). Further studies in mice regarding a possible vaccine against Bilharziasis (*Schistosoma mansoni*). *Boletin de la Association Medica de Puerto Rico* **68**, 312–313.

Rhodes, M. B., Nayak, D. P., Kelley, G. W. and Marsh, C. L. (1965). Studies on Helminth enzymology. IV. Immune response to malic dehydrogenase from *Ascaris suum*. *Experimental Parasitology* **16**, 373–381.

Rhodes, M. B., Marsh, C. L. and Ferguson, D. L. (1969). *Ascaris suum:* purification and characterisation of an intestinal aminopeptidase. *Experimental Parasitology* **26**, 150–155.

Rickard, M. D. and Adolph, A. J. (1976). Vaccination of calves against *Taenia saginata* infection using a 'parasite-free' vaccine. *Veterinary Parasitology* **1**, 389–392.

Rickard, M. D. and Arundel, J. H. (1974). Passive protection of lambs against infection with *Taenia ovis* via colostrum. *Australian Veterinary Journal* **50**, 22–24.

Rickard, M. D. and Bell, K. J. (1971a). Immunity produced against *Taenia ovis* and *T. taeniaeformis* infection in lambs and rats following *in vivo* growth of their larvae in filtration membrane diffusion chambers. *Journal of Parasitology* **57**, 571–575.

Rickard, M. D. and Bell, K. J. (1971b). Successful vaccination of lambs against infection with *Taenia ovis* using antigens produced during *in vitro* cultivation of the larval stages. *Research in Veterinary Science* **12**, 401–402.

Rickard, M. D. and Katiyar, J. C. (1976). Partial purification of antigens collected *in vitro* cultivation of the larval stages of *Taenia pisiformis*. *Parasitology* **72**, 269–279.

Rickard, M. D. and Outteridge, P. M. (1974). Antibody and cell-mediated immunity in rabbits infected with the larval stages of *Taenia pisiformis*. *Zeitschrift für Parasitenkunde* **44**, 187–201.

Rickard, M. D., White, J. B. and Boddington, E. B. (1976). Vaccination of lambs against infection with *Taenia ovis*. *Australian Veterinary Journal* **52**, 209–214.

Ritchie, L. S., Garson, S. and Erickson, D. G. (1962). Attempts to induce resistance against *Schistosoma mansoni* by injecting cercarial, adult worm and egg homogenates in sequence. *Journal of Parasitology* **48**, 233–236.

Ross, J. G. (1967). Studies of immunity to *Fasciola hepatica:* acquired immunity in cattle, sheep and rabbits following natural infection and vaccine procedures. *Journal of Helminthology* **41**, 393–399.

Rothwell, T. L. W. and Love, R. J. (1974). Vaccination against the nematode *Trichostrongylus colubriformis.* I. Vaccination of guinea-pigs with worm homogenates and soluble products released during *in vitro* maintenance. *International Journal for Parasitology* **4**, 293–299.

Rothwell, T. L. W. and Merritt, G. C. (1975). Vaccination against the nematode *Trichostrongylus colubriformis.* II. Attempts to protect guinea-pigs with worm acetylcholinesterase. *International Journal for Parasitology* **5**, 453–460.

Sadun, E. H. and Bruce, J. I. (1964). Resistance induced in rats by previous exposure to and by vaccination with fresh homogenates of *Schistosoma mansoni. Experimental Parasitology* **15**, 32–43.

Sadun, E. H. and Lin, S. S. (1959). Studies on the host parasite relationship to *Schistosoma japonicum.* IV. Resistance acquired by infection, by vaccination and by the injection of immune serum, in monkeys, rabbits and mice. *Journal of Parasitology* **45**, 543–548.

Sanderson, B. E. and Ogilvie, B. M. (1971). A study of acetylcholinesterase throughout the life cycle of *Nippostrongylus brasiliensis. Parasitology* **62**, 367–373.

Sarles, M. P. (1938). The *in vitro* action of immune rat serum on the nematode, *Nippostrongylus muris. Journal of Infectious Diseases* **62**, 337–348.

Sen, H. G. (1972). *Necator americanus:* behaviour in hamsters. *Experimental Parasitology* **32**, 26–32.

Senft, A. and Senft, D. (1962). A chemically defined medium for maintenance of *Schistosoma mansoni. Journal of Parasitology* **48**, 551–554.

Sheldon, A. J. (1937). Studies on active acquired resistance, natural and artificial, in the rat to infection with *Strongyloides ratti. American Journal of Hygiene* **25**, 53–65.

Sher, F. A., Kusel, J. R., Perez, H. and Clegg, J. A. (1974). Partial isolation of a membrane antigen which induces the formation of antibodies lethal to schistosomes cultured *in vitro. Clinical Experimental Immunology* **18**, 357–369.

Shikhobalova, N. P. (1953). Experimental tests on immunity in trichinelliasis. III. Immunity acquired as a result of injections (In Russian). *Izdatelstvo Akademii Nauk SSSR*, 761–769.

Silva, da. L. and Ferri, R. G. (1968). *Schistosoma mansoni* homogenate for active immunization of mice. *American Journal of Tropical Medicine and Hygiene* **17**, 369–371.

Silverman, P. H. (1959). *In vitro* cultivation of the histotrophic stages of *Haemonchus cortortus* and *Ostertagia* spp. *Nature, London* **183**, 197.

Silverman, P. H. (1965). *In vitro* cultivation procedures for parasitic helminths. *In* "Advances in Parasitology," volume 3 (B. Dawes, ed.) pp. 159–222. Academic Press, New York and London.

Silverman, P. H. (1970). Vaccination: Progress and Problems. *In* "Immunity to Parasitic Animals," volume 2 (G. J. Jackson, R. Herman and I. Singer, eds) pp. 1165–1185. Appleton-Century-Crofts, New York.

Silverman, P. H. and Hansen, E. L. (1971). *In vitro* cultivation procedures for parasitic helminths: recent advances. *In* "Advances in Parasitology," volume 9 (B. Dawes, ed.) pp. 227–258. Academic Press, New York and London.

Silverman, P. H., Poynter, D. and Podger, K. R. (1962). Studies on larval antigens derived by cultivation of some parasitic nematodes in simple media: protection tests in laboratory animals. *Journal of Parasitology* **48**, 562–561.

Sinclair, I. J. and Joyner, L. P. (1974). The effect of the administration of a homologous antigen on the establishment of *Fasciola hepatica* in the rabbit. *Research in Veterinary Science* **16**, 320–327.

Smith, M. A., Clegg, J. A., Kusel, J. R. and Webbe, G. (1975). Lung inflammation in immunity to *Schistosoma mansoni*. *Experientia* **31**, 595–596.

Smithers, S. R. (1962). Stimulation of acquired resistance to *Schistosoma mansoni* in monkeys: role of eggs and worms. *Experimental Parasitology* **12**, 263–273.

Smithers, S. R. (1976). Immunity to trematode infections with special reference to schistosomiasis and fascioliasis. *In* "Immunology of Parasitic Infections" (S. Cohen and E. Sadun, eds.) pp. 296–332. Blackwell Scientific Publications, Oxford.

Smithers, S. R. and Terry, R. J. (1967). Resistance to experimental infection with *Schistosoma mansoni* in rhesus monkeys induced by the transfer of adult worms. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **61**, 517–533.

Smithers, S. R. and Terry, R. J. (1969). The immunology of schistosomiasis. *In* "Advances in Parasitology," volume 7 (B. Dawes, ed.) pp. 41–93. Academic Press, New York and London.

Smithers, S. R. and Terry, R. J. (1976). The immunology of schistosomiasis. *In* "Advances in Parasitology," volume 14 (B. Dawes, ed.) pp. 399–422. Academic Press, New York and London.

Smyth, J. D. (1967). Studies on tapeworm physiology. IX. *In vitro* cultivation of *Echinococcus granulosus* from the protoscolex to the strobilate stage. *Parasitology* **57**, 111–113.

Smyth, J. D. (1969). The biology of the hydatid organisms. *In* "Advances in Parasitology," volume 7 (B. Dawes, ed.) pp. 327–347. Academic Press, New York and London.

Smyth, J. D. (1976). "Introduction to Animal Parasitology", 2nd edition, pp. 423–446. Hodder and Stoughton, London.

Soulsby, E. J. L. (1961). Some aspects of the mechanism of immunity to helminths. *Journal of the American Veterinary and Medical Association* **138**, 355–362.

Soulsby, E. J. L. (1963). The nature and origin of the functional antigens in helminth infections. *Annals of the New York Academy of Science* **113**, 492–509.

Soulsby, E. J. L. (1966). The mechanisms of immunity to gastro-intestinal nematodes. *In* "Biology of Parasites" (E. J. L. Soulsby, ed.) pp. 255–273. Academic Press, New York and London.

Soulsby, E. J. L. and Stewart, D. F. (1960). Serological studies of the 'self-cure' reaction in sheep infected with *Haemonchus contortus*. *Australian Journal of Agricultural Research* **11**, 595–603.

Spindler, L. A. (1937). Resistance to intestinal trichinosis in experimental animals induced by feeding metabolic products of encysted trichinae. *Journal of the Washington Academy of Sciences* **27**, 36–38.

Sprent, J. F. A. and Chen, H. H. (1949). Immunological studies in mice infected with larvae of *Ascaris lumbricoides*. I. Criteria of immunity and the immunizing effect of isolated worm tissues. *Journal of Infectious Diseases* **84**, 111–124.

Stewart, D. F. (1953). Studies on resistance of sheep to infestation with *Haemonchus contortus* and *Trichostrongylus* species and on the immunological reactions of sheep exposed to infestation. V. The nature of the self-cure phenomenon. *Australian Journal of Agricultural Research* **4**, 100–117.

Stoll, N. R. (1929). Studies with the *strongyloid* nematode, *Haemonchus contortus*. I. Acquired resistance of hosts under natural reinfection conditions out-of-doors. *American Journal of Hygiene* **10**, 384–418.

Stoll, N. R. (1947). This wormy world. *Journal of Parasitology* **33**, 1–18.
Stoll, N. R. (1961). The worms: can we vaccinate against them? *American Journal of Tropical Medicine and Hygiene* **10**, 293–303.
Taffs, L. F. (1968). Immunological studies on experimental infection of pigs with *Ascaris suum* Goeze, 1782. VI. The histopathology of the liver and lung. *Journal of Helminthology* **42**, 157–172.
Taliaferro, W. H. and Sarles, M. P. (1939). The cellular reactions in the skin, lungs and intestine of normal and immune rats after infection with *Nippostrongylus muris*. *Journal of Infectious Diseases* **64**, 157–192.
Taylor, A. E. R. and Baker, J. R. (1968). "The cultivation of parasites *in vitro*". Blackwell Scientific Publications, Oxford.
Taylor, M. G., James, E. R., Nelson, G. S., Bickle, Q. Dunne, D. W. and Webbe, G. (1976). Immunisation of sheep against *Schistosoma mattheei* using either irradiated cercariae or irradiated schistosomula. *Journal of Helminthology* **50**, 1–9.
Thompson, J. H. (1954). Host-parasite relationships of *Schistosoma mansoni*. *Experimental Parasitology* **3**, 140–160.
Thompson, R. C. A. (1976). Inhibitory effect of BCG on development of secondary hydatid cysts of *Echinococcus granulosus*. *Veterinary Record* **99**, 273.
Thorson, R. E. (1953). Studies on the mechanism of immunity in the rat to the nematode *Nippostrongylus muris*. *American Journal of Hygiene* **58**, 1–15.
Thorson, R. E. (1954). Absorption of protective antibodies from serum of rats immune to the nematode *Nippostrongylus muris*. *Journal of Parasitology* **40**, 300–303.
Thorson, R. E. (1956a). Proteolytic activity in extracts of the oesophagus of adults of *Ancylostoma caninum* and the effect of immune serum on this activity. *Journal of Parasitology* **42**, 21–25.
Thorson, R. E. (1956b). The stimulation of acquired immunity in dogs by injection of extracts of the oesophagus of adult hookworms. *Journal of Parasitology* **42**, 501–504.
Thorson, R. E. (1963). Seminar on immunity to parasitic helminths. II. Physiology of immunity to helminth infections. *Experimental Parasitology* **13**, 3–12.
Thorson, R. E. (1970). Direct infection nematodes. *In* "Immunity to Parasitic Animals, Volume 2" (G. J. Jackson, R. Herman and I. Singer, eds) pp. 913–961. Appleton-Century-Crofts, New York.
Tropical Health. (1962). "A report on a study of needs and resources". National Academy of Sciences, National Research Council, Washington, D.C.
Turner, E. L., Berberian, D. A. and Dennis, E. W. (1933). Successful artificial immunization of dogs against *Taenia echinococcus*. *Proceedings of the Society of Experimental biology and Medicine* (*New York*) **30**, 618–619.
Turner, E. L., Berberian, D. A. and Dennis, E. W. (1936). The production of artificial immunity in dogs against *Echinococcus granulosus*. *Journal of Parasitology* **22**, 14–28.
Urquhart, G. M. (1961). Epizootiological and experimental studies on bovine *cysticercosis* in East Africa. *Journal of Parasitology* **47**, 857–869.
Urquhart, G. M., Mulligan, W. and Jennings, F. W. (1954). Artificial immunity to *Fasciola hepatica* in rabbits. I. Some studies with protein antigens of *F. hepatica*. *Journal of Infectious Diseases* **94**, 126–133.
Urquhart, G. M., Jarrett, W. F. H., Jennings, F. W., McIntyre, W. I. M. and Mulligan, W. (1966). Immunity to *Haemonchus contortus:* relationship between age and successful vaccination with irradiated larvae. *American Journal of Veterinary Research* **27**, 1645–1647.

Van Fleteren, J. R. (1976). Large-scale cultivation of a free-living nematode (*Caenorhabditis elegans*). *Experientia* **32**, 1087–1088.

Vogel, H. (1949). Immunologie der helminthiasen. *Zentralblatt für Bakteriologie Parasitenkunde, Infektionskrankheiten und Hygiene* **154**, 118–126.

Vogel, H. and Minning, W. (1953). Uber die erworbene resistang von *Macacus rhesus* gegenuber *Schistosoma japonicum. Zeitschrift für Tropenmedizin und Parasitologie* **4**, 418–505.

Wakelin, D. and Selby, G. R. (1973). Functional antigens of *Trichuris muris*. The stimulation of immunity by vaccination of mice with somatic antigen preparations. *International Journal for Parasitology* **3**, 711–715.

Watt, J. Y. C. (1943). Active immunization of rats against *Nippostrongylus muris*. *Proceedings of the Society for Experimental Biology and Medicine* **52**, 67–72.

Watts, N. P. (1949). Prophylactic use of schistosomal antigen. *Journal of Immunology* **62**, 183–192.

Weinmann, C. J. (1970). Cestodes and Acanthocephala. *In* "Immunity to parasitic animals", volume 2 (G.J. Jackson, R. Herman and I. Singer, eds pp. 1021–1059. Appleton-Century-Crofts, New York.

Weller, T. H. (1976). Manson's schistosomiasis; frontiers *in vivo, in vitro,* and in the body politic. *American Journal of Tropical Medicine and Hygiene* **25**, 208–216.

World Health Organization (1974a). Expert committee on filariasis, 3rd report. *Technical Report Series* **542**, 1–54.

World Health Organization. (1974b). Immunology of schistosomiasis. *Bulletin of the World Health Organization* **51**, 553–595.

World Health Organization. (1976a). Aspects of international health work in 1975. *Chronicle of the World Health Organization* **30**, 264–285.

World Health Organization. (1976b). Immunological adjuvants. *Technical Report Series* **595**, 1–40.

Genetic Control of Susceptibility and Resistance to Parasitic Infection

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I. Introduction

The susceptibility or resistance of a host species to parasitic infection is the product of a variety of factors which influence the host–parasite relationship at many points (Sprent, 1969). The possibility of contact between parasite and host is regulated by ecological and behavioural factors, but once contact is made, the outcome of infection is governed by factors arising from the innate and acquired characteristics of the host. Since such characteristics are genetically controlled, it follows that susceptibility and resistance at the species level can also be genetically determined. Although not always formulated in these terms the idea of interspecific variation is a familiar one in parasitology,

finding expression in two concepts, that of the host specificity of parasites, and that of the parasitocenose, the parasite fauna characteristic of a given host species (Pavlovsky, 1966). Specificity of parasites can be seen as the evolutionary consequence of host genetic diversity, the parasitocenose as the assemblage of parasites which have become adapted for life in the environment specified by the genotype of a particular host species. Interspecific variation of this kind is paralleled by intraspecific variation, the existence of genetically determined variation in susceptibility or resistance to parasites for which the species is a natural host. Such intraspecific variation has been recognized for many years in man and domestic animals, but the wider occurrence of the phenomenon has received relatively little attention in parasitology and has certainly not been investigated experimentally to the extent that it has in other areas of infectious disease. To some degree the phenomenon has been obscured by a lack of understanding of the factors which determine the outcome of parasitic infections, since analysis of the interactions of complex parasites with complex hosts presents particularly difficult problems. Nevertheless, it is probably not an exaggeration to say that failure adequately to recognize intraspecific variability in response to infection has hindered experimental investigation of the host–parasite relationship and may have delayed the development of effective control measures against economically important species.

This review will be restricted to consideration of examples of genetically-controlled variation in response to parasites operating at the intraspecific level, because such variations offer, through comparison of infections in different host strains and by selective breeding programmes, scope for experimental investigation of the mechanisms responsible; variation associated with differences in age and sex will not be considered. The review will deal only with parasites in animal hosts; the genetics of plant host–parasite relationships, though providing many interesting and significant analogies, have been amply covered in a number of recent reviews (see Webster, 1975). The majority of examples will be taken from infections in vertebrate (avian and mammalian) hosts since, although there is a substantial body of literature dealing with the genetics of susceptibility and resistance to parasitic infection in invertebrates, little is understood of the means by which genetic control is mediated. Emphasis will be given to examples where susceptibility and resistance have been or could reasonably be interpreted in relation to the operation of immunological responses imposing restraints upon the development and persistance of parasites. The rapid growth of immunogenetics during the last fifteen years has provided a rational basis for the investigation and explanation of many aspects of intraspecific variation in response to infection in vertebrate hosts. It is now well established that, although immunity as such is not inherited, the capacity to respond immunologically to particular antigens is in many cases under direct and relatively simple genetic control. Thus immunologically mediated resistance to an infectious organism may be genetically determined and show qualitative and quantitative variation with the genetic diversity that exists between individuals and between strains of a host species. Recognition of this fact in other fields of infectious disease, notably virology (see Allison,

1965; McDevitt *et al.*, 1974), has stimulated many rewarding analyses of the host–parasite relationship, the aetiology of disease and the nature of resistance to infection. It might be thought that, since immune responses to parasitic animals are invariably complex, the existence of genetically controlled variations in immunologically-mediated resistance to infection would be difficult to demonstrate. Though this may sometimes be true, it is perhaps more likely that the complexity of response is irrelevant, many components of the response having little or no effect upon the parasite. Effective immune responses, i.e. those imposing some restraint upon the parasite, are likely to be mediated by only a few components of the response and thus genetic control should be demonstrable. Recognition of this fact offers the possibility of an exciting re-evaluation of the response of hosts to parasitic infection and may allow an analysis of the immune response in terms which clarify both the underlying genetic and immunological mechanisms. Such information will be of considerable value in the field of parasite control, in elucidating some of the pathological consequences of infection and in clarifying the ecological interactions of hosts and parasites.

II. Susceptibility, Resistance and Modes of Inheritance

The terms "susceptibility" and "resistance" are notoriously difficult to define precisely and they have been used in a variety of senses by different authors (see Read, 1958; Sprent, 1969). Susceptibility carries the implication that, in a particular host–parasite combination, the host is capable of providing an environment in which establishment, development and maturation of the parasite are possible. Resistance implies that there are attributes of the host, both innate and acquired, which can impose limitations upon the parasite at any stage of its relationship with the host. Susceptibility and resistance may be absolute; in the context of intraspecific variation they are more frequently relative. Relative susceptibility carries the corollary of relative resistance and for convenience, and brevity, susceptibility will be used here as a comprehensive term denoting the overall suitability of a host for a parasite.

Intraspecific variation in a particular characteristic is essentially of two types, discontinuous and continuous. Discontinuous variation is evident in a population when individuals can be assigned to two or more clearly defined phenotypes and it normally reflects control by a single gene or by a small number of genes. Continuous variation, in which individual phenotypes cannot be sharply demarcated, usually implies control by a large number of genes, polygenes, but may also arise when the action of a small number of genes is modified by strong non-genetic factors. Discontinuous variation is, of course, easier to detect in a population and is more amenable to experimental investigation. The nature of the genetic control underlying a particular variation can be analysed by breeding experiments, commonly by testing the progeny of a cross between parents of opposite phenotype (the F_1 generation) and those of crosses between F_1 and parental individuals (the backcross). The distribution of susceptibility and resistance in populations showing discontinuous variation under single major gene control or continuous variation

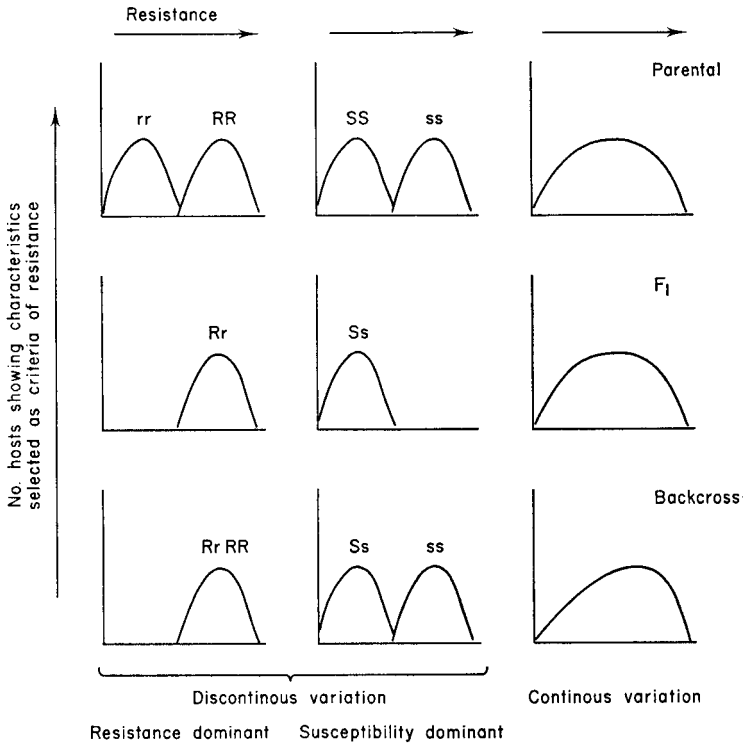

FIG. 1. Inheritance of susceptibility and resistance in progeny derived by crossing parents of opposite phenotype (the F_1 generation) and by backcrossing F_1 individuals to parents of resistant phenotype.

under polygenic control is shown diagrammatically in Fig. 1, together with the patterns of inheritance that would be expected in the F_1 and from the backcross to the parent showing resistant phenotype. For simplicity the illustration assumes homozygosity in the parental types showing discontinuous variation (as for example in inbred strains); in practice, one parental type may be heterozygous, in which case the F_1 and backcross results will differ from those shown. When discontinuous variation is controlled by more than one gene the F_1 generation from a cross made between susceptible and resistant parents would be predominantly as shown in Fig. 1, but the backcross generation would show divergence from the pattern shown, the ratio of phenotypes in the backcross generation allowing some conclusions to be drawn about the number of genes involved.

III. INVERTEBRATE HOST–PARASITE RELATIONSHIPS

Almost all the information available on intraspecific variation in response to parasitic infection has come from studies of three medically important

host–parasite relationships, namely, malarial parasites in mosquitoes, filariid nematodes in mosquitoes and schistosomes in snails (Table 1). The impetus for much of this work stems from the realization that an understanding of the genetic basis of resistance to infection may allow biological control methods to be devised (Pal, 1967; Richards, 1970; Macdonald, 1976).

TABLE 1

Invertebrate host-parasite relationships in which genetically determined intraspecific variation in susceptibility and resistance has been demonstrated

Parasite	*Host*	*Reference*
Plasmodium cathemerium	*Culex pipiens*	Huff, 1929, 1931
P. elongatum	*Culex pipiens*	Micks, 1949
P. lophurae	*Aedes aegypti*	Trager, 1942
P. gallinaceum	*Aedes aegypti*	Ward 1963; Kilama and Craig, 1969
P. gallinaceum	*Anopheles quadrimaculatus*	Rockefeller Foundation, 1950
Schistosoma mansoni	*Biomphalaria glabratus*	See text
S. rodhaini	*Biomphalaria glabratus*	Saoud, 1966
S. japonicum	*Oncomelania formosana*	Moose and Williams, 1963
Fasciola gigantica	*Lymnaea auricularia*	Kendall and Parfitt, 1959
Spirometra mansonoides	*Cyclops vernalis*	Mueller, 1966
Brugia malayi	*Aedes aegypti*	Macdonald, 1962a,b; 1963
Dirofilaria immitis	*Aedes aegypti*	Kartman, 1953; McGreevy *et al.*, 1974
Wuchereria bancrofti	*Psorophora confinnis*	Newton *et al.*, 1945
W. bancrofti	*Aedes aegypti*	Macdonald and
Brugia pahangi	*Aedes aegypti*	Ramachandran, 1965
B. pahangi	*Culex pipiens*	Obiamiwe and Macdonald, 1973
Nasonia vitripennis	*Musca domestica*	Olson and Pimental, 1974

A. MALARIA AND MOSQUITOES

It has long been recognized that within populations of malaria-transmitting mosquitoes there is considerable individual variation in ability to transmit infection, ranging from complete susceptibility and successful transmission to complete resistance (refractoriness). The suggestion that such variation had a genetic basis was first made by Huff (1929) working with *Culex pipiens* and *Plasmodium cathemerium*. By selective breeding Huff was able in three generations to increase the proportion of suceptible mosquitoes in his stock from 28% to 65% and to decrease the proportion of refractory mosquitoes to

7·5%. In later work (Huff, 1931), the genetic mechanisms underlying the variation were analysed and Huff concluded that susceptibility was controlled by a pair of alleles acting in a simple Mendelian recessive fashion. More recently, however, Kilama and Craig (1969) have questioned this conclusion since Huff's backcross ratios showed significant divergence from a 1 : 1 ratio.

Other workers have carried out similar breeding experiments with other mosquito–malaria combinations and have shown that susceptibility and refractoriness are genetically determined, but in only a few cases has the mode of inheritance been analysed. The susceptibility of *Aedes aegypti* to *Plasmodium gallinaceum* has been studied by Ward (1963) and by Kilama and Craig (1969) who considered that susceptibility was controlled by a dominant autosomal allele which they termed *pls* (= plasmodium susceptibility). Kilama and Craig surveyed 19 strains of *A. aegypti* and found wide variation in susceptibility to infection. They established within one generation two refractory strains in which oocyst formation was virtually absent, susceptible strains allowing development of more than 50 oocysts.

The factors which determine the success or failure of malarial infections in mosquitoes are not understood and the processes controlled by the genes concerned are unknown. Susceptibility may reflect the availability in the host of metabolic factors essential for the normal development of the parasite. Clark and Ball (1952) showed the presence of up to 20 amino acids in whole body extracts of culicid mosquitoes and suggested that variation in these amino acids may account for variations in susceptibility to malaria between different species. Kilama and Craig (1969) proposed that the gene *pls*, which determined susceptibility of *A. aegypti* to *P. gallinaceum*, controlled the production of factors necessary for oocyst development. Certainly it has been shown that the susceptibility of mosquitoes to infection can be altered by alteration of dietary constituents, e.g. feeding para-amino-benzoic acid altered the susceptibility of both susceptible *A. aegypti* and refractory *Culex pipiens pipiens* to infection with *P. gallinaceum* (Noblet and Weathersby, 1973). Alternatively, refractoriness may be the result of defensive reactions to the invading parasite. Mosquitoes, like some other Diptera, are relatively deficient in haemocytes and the protective response may therefore involve humoral rather than cellular mechanisms (Weathersby, 1960; Weathersby and McCall, 1968).

B. FILARIIDS AND MOSQUITOES

As with malaria parasites, it was shown some time ago (Kartman, 1953; Macdonald, 1962a) that selective breeding could increase or decrease the proportion of individuals susceptible to filariid nematode infection, for example, Macdonald increased the proportion of *Aedes aegypti* susceptible to subperiodic *Brugia malayi* from 17% to 90% in one generation by breeding from susceptible parents, the increased susceptibility remaining constant for 15 generations (Table 2). Analysis of the genetic basis involved was made by Macdonald (1962b, 1963) who showed that susceptibility was controlled by a single sex-linked, recessive gene designated f^m (= filarial susceptibility

TABLE 2

The results of feeding 15 generations of a selected strain of Aedes aegypti *on cats infected with semi-periodic* Brugia malayi
(*Modified from McDonald, 1961a*)

Generation	No. of mosquitoes dissected	% of mosquitoes with mature larvae	No. of mature larva per infective mosquito
Parent	105	17·1	4·0
F_1	32	93·8	6·5
F_3	14	85·7	9·2
F_5	68	88·2	7·0
F_7	32	90·6	4·7
F_9	30	80·0	3·4
F_{11}	15	93·3	4·9
F_{15}	39	84·6	5·1

(*Brugia malayi*)). This gene showed incomplete penetrance, i.e. its expression could be modified by other genes so that the characteristic of susceptibility was not always evident in individuals carrying f^m. Subperiodic *B. malayi* develops in the thoracic muscles of the mosquito and Macdonald and Ramachandran (1965) have shown that the gene f^m also controls the susceptibility of *A. aegypti* to other filariids developing in this site (periodic *B. malayi*, *B. pahangi*, periodic and subperiodic *Wuchereria bancrofti*) but had no effect on susceptibility to filariids which developed in the Malpighian tubules (*Dirofilaria immitis* and *D. repens*). Barr (1975) concluded that the degree of susceptibility in Macdonald's laboratory strain was unnatural and bore little relation to the susceptibility of natural vectors, which is normally limited to single filariids.

McGreevy *et al.* (1974) have identified an additional gene, *ft*, in *A. aegypti*, which controls susceptibility to *Dirofilaria immitis*. They found that this gene had no control over the development of another species, *D. corynodes*, which develops in the fat body of the insect and concluded that susceptibility to filarial species in *A. aegypti* is controlled by genes "that directly affect the organ lodging the parasite rather than the parasite itself". This idea of a local influence contradicts Barr's view (1975) that a more general, basic defence mechanism is involved, but the nature of the response is unknown. Beckett and Macdonald (1971) showed that, even in susceptible *A. aegypti*, considerable numbers of *B. malayi* larvae died before leaving the thoracic muscles, supporting the idea of a localized tissue response, but evidence has been presented by other workers (Esslinger, 1962; Poinar, 1969) that humoral responses can also kill filariids in mosquitoes. Desowitz and Challappah (1965) made the interesting observation that transplanted third-stage larvae of *B. pahangi* survived equally well in susceptible and refractory hosts, suggesting that host specificity operates most effectively during the initial and developmental stages of infection. As is the case with mosquito susceptibility to

malaria, it may be that both defensive responses and physiological characteristics contribute to susceptibility or resistance to filarial infection. It has recently been shown, for example, (see Macdonald, 1976) that *A. aegypti* susceptible to *D. immitis* and *D. repens* show high levels of alkaline phosphatase activity.

Townson (1971) showed that infection of *A. aegypti* by *B. malayi* caused some mortality in the mosquitoes, both refractory and susceptible. Two peaks of mortality occurred, one within the first 24 hours after the infective blood meal and one six to ten days later, the latter occurring only in susceptible mosquitoes. The persistence of the susceptibility genotype in populations may therefore arise from some heterozygote advantage. Rodriguez and Craig (1973) raised the possibility that susceptibility to filarial infection may at one time have been widespread in populations of *A. aegypti*, but that the mortality associated with heavy infection may have favoured the emergence of refractory genotypes. *A. aegypti* is not an important vector of human filariasis but Rodriguez and Craig suggested that human filariasis may have played a major role in selecting for refractory genotypes in this species and supported their view by evidence that strains from endemic areas were wholly or almost wholly refractory to experimental infection with *Brugia pahangi*.

The infectivity of filariids for mosquitoes has been analysed most fully in *A. aegypti*, but there is evidence for similar genetically-determined variation in at least one other species, *Culex pipiens*. Obiamiwe and Macdonald (1973) have shown evidence for a sex-linked recessive gene, *sb*, which controls susceptibility to *Brugia pahangi* in this host, but the gene has no effect upon susceptibility to *Wuchereria bancrofti* for which *C. pipiens* is a natural vector.

C. SCHISTOSOMES AND SNAILS

Digenea as a class show a high degree of specificity for the molluscan intermediate host. In certain cases, and particularly in schistosomes, this specificity extends to intraspecific levels, compatibility existing only between parasites and hosts from the same endemic area (Files and Cram, 1949; Files, 1951; Barbosa and Barreto, 1960). Newton (1952) working with *Schistosoma mansoni* and *Biomphalaria glabrata* of different strains showed that both susceptible (Puerto Rican) and refractory (Brazilian) snails were penetrated by miracidia, but found that the latter hosts responded rapidly to infection, a marked cellular infiltration surrounding and destroying the parasite in its early developmental stages. By cross-breeding the two strains Newton (1953) found that susceptibility to infection was genetically determined, a number of genetic factors being involved.

More detailed genetic analysis of the susceptibility of *B. glabrata* to *S. mansoni* has been carried out by Richards and it is apparent that, in juvenile snails, susceptibility is controlled by a complex of at least four genetic factors (Richards and Merritt, 1972). Richards used cloning techniques, deriving his snail populations from self-fertilized parental snails, and was able to establish clonal stocks breeding true for either 100% or 0% susceptibility, However.

because of the polygenic nature of the control involved, snails whose self-fertilized progeny bred true, could produce progeny of the opposite susceptibility when crossed. Richards (1970) observed, as had Newton (1953) earlier, that some of the stocks of snails which showed susceptibility as juveniles became refractory to infection as adults. This refractoriness was, in some populations, determined by a single dominant gene (Richards, 1973). No comparable change in susceptibility was seen in stocks that were refractory as juveniles, but an additional complication was the finding that some stocks that were susceptible as juveniles and refractory as adults, reverted to susceptibility in old age (Fig. 2).

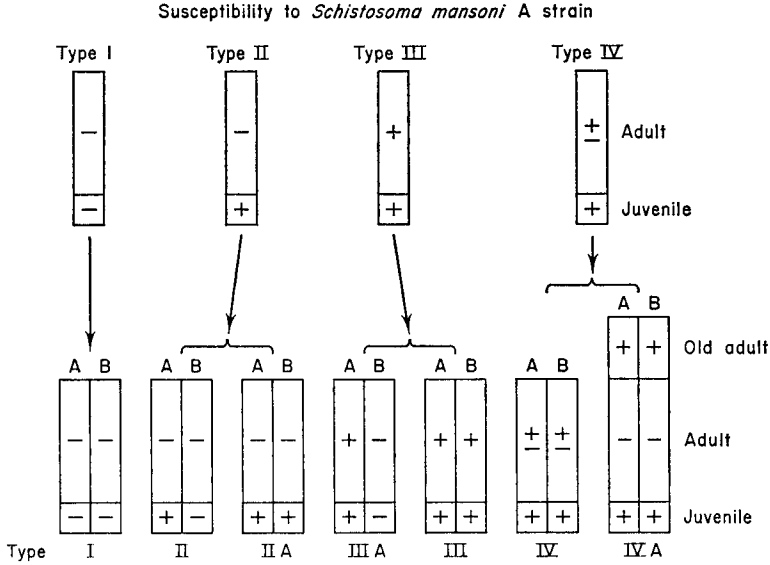

FIG. 2. Genetic variations in susceptibility of juvenile and adult *Biomphalaria glabrata* to infection with two strains of *Schistosoma mansoni*. (A from Puerto Rico, B from St. Lucia). Susceptible = +, Refractory = —, Variable susceptibility = ±. (Modified from Richards, 1975)

There is as yet no clear interpretation of the nature of the response controlled by the genes determining susceptibility to schistosome infection. From the work of Newton (1952) and others (Pan, 1963; Tripp, 1963) it would appear that some effect upon the interaction of amoebocytes with the parasite is likely to be involved, since such cells are the major effectors of defensive responses against parasites in snails, but it is not possible to say at what level the control is exerted.

Although intraspecific variation has been studied most extensively in snails infected with *S. mansoni*, evidence exists that genetically determined differences in susceptibility also occur in the molluscan hosts of *S. japonicum*, *S. haematobium* (DeWitt, 1954; Moose and Williams, 1963) and *S. rodhaini*

(Saoud, 1966). In addition, Kendall and Parfitt (1959) have described differences in susceptibility of strains of *Lymnaea truncatula* to *Fasciola gigantica*.

Kagan and Geiger (1965) presented evidence that the susceptibility of snails to *S. mansoni* was a relative phenomenon and had to be considered in the context of genetic variation in both snail and parasite. They showed that refractory snails could be infected by more invasive miracidia and that selection of miracidia occurred as a result of passage through the host, concluding, "We believe that the snail host is potentially susceptible to infection and the genetics of the miracidium is the deciding variable in this host–parasite relationship". The question of "snail susceptibility or trematode infectivity" has also been posed by Wright (1974). On the basis of a mathematical analysis of snail–miracidium interaction Dönges (1974) concluded that snail populations were homogeneous with respect to susceptibility to infection, with no appreciable proportion of resistant individuals. Basch (1975, 1976) has disagreed with this interpretation and proposed that specificity in the snail–parasite relationship is based upon genetically determined characters of compatibility in both members, individual snails being resistant or susceptible to individual miracidia.

D. OTHER INVERTEBRATE HOST–PARASITE RELATIONSHIPS

Although the three relationships described above are the best documented examples of genetic control of susceptibility to parasites in invertebrates, there are a few other examples known. Mueller (1966) found that with repeated infection of a breeding population of *Cyclops vernalis* the proportion of individuals initially infected with oncospheres of *Spirometra mansonoides* fell from 70% to 10% and the proportion harbouring mature procercoids from 30–40% to 1% or less. Mueller concluded that "it seems difficult to explain this result on any basis except that susceptibility is a genetic trait". The basis of the selection exerted by the parasite was the failure of infected copepods to reproduce, the regenerating population being derived from refractory individuals. Although the mechanism of resistance was not elucidated Mueller suggested that copepods had two lines of defence against infection and could destroy invading parasites either in the gut or in the haemocoel.

Genetically controlled resistance of insects to parasitoids has also been demonstrated (Olson and Pimental, 1974), the parasitoid selecting, by virtue of its destruction of reproductive stages, those members of the population which were more resistant to the invasive stages.

It is possible that intraspecific variation is a widespread phenomenon in invertebrate host–parasite relationships and that the paucity of examples arises from insufficiently detailed examination. The work of Hynes and Nicholas (1958) on the success or failure of larval development of *Polymorphus minutus* in three closely related species of *Gammarus* suggests very strongly that intraspecific variation would be detectable if looked for. Their observations showed that, although development of *P. minutus* was com-

pleted in the majority of homologous gammarids infected (i.e. the species in which the egg-producing adult worms had themselves undergone larval development), the infection effectively sterilized the amphipods. It is very probable that in a closed breeding population such selection would reveal the presence of resistant individuals as Mueller (1966) found with *Cyclops* and *Spirometra*. Variability of development within invertebrate intermediate host populations is well documented in two major diseases of man, namely onchocerciasis and trypanosomiasis, although there has been no formal demonstration of a genetic basis for the variation. Duke *et al.* (1966) showed that there was a complex interrelationship between *Onchocerca volvulus* and *Simulium damnosum* in West Africa. Strains of the parasite from the Cameroon forest area developed well in *Simulium* from forest and Guinea savanna zones, but showed little development in flies from Sudan-savanna zones. This interrelationship probably involves variation in both parasite and vector. Infection of *Glossina* sp. with trypanosomes is notoriously variable and overall infection rates within a population are usually low. A number of factors associated with the source of infection, the environment and the development of the fly are known to influence infection rates (Wijers, 1958) but under standardized conditions it has been shown that the percentage of flies acquiring established infections is a characteristic of the fly population concerned (Dipeolu and Adam, 1974). The proportion of established infections which matured was variable and some of the variability arose from environmental factors affecting the fly. However, Brown (1976) has suggested that there may be genetically determined epithelial cell configurations which influence the contact of trypanosomes with tsetse salivary gland epithelial cells and thus control the degree of multiplication and redifferentiation that can occur.

IV. Vertebrate Host—Parasite Relationships: Genetic Control Mediated via Innate Host Characteristics

Susceptibility or resistance to parasitic infections in vertebrate hosts is determined by a spectrum of factors operating independently or in concert. By choosing examples from each end of the spectrum it is possible to categorize such factors into those which are a consequence of the host's structural, biochemical and physiological make-up and which exist prior to contact with the parasite, and those which arise as a result of defence responses and other changes consequent upon experience of infection. These categories correspond to the "suitability" and "resistance" factors of Sprent (1969) or more approximately with "innate" and "acquired" immunity respectively. Many of the reported instances of intraspecific variation do not allow an unambiguous decision to be made as to the nature of the factors responsible and indeed factors from both categories may be involved. Nevertheless, some division is possible and necessary, therefore for convenience, the examples which follow will be considered under the heading of genetic control mediated via innate characteristics. Examples of genetic control acting via acquired characteristics will be considered in section VI.

A. PROTOZOAN PARASITES

Parasitic organisms are intimately affected by the physiology of their hosts at all stages of their parasitic existence and it is widely recognized that physiological compatibility between parasite and host at the species level is a major determinant of natural resistance to infection. It is likely that many of the physico-chemical factors which determine susceptibility to infection will show intraspecific variation and that this will be reflected in variability of parasite development between individual hosts of the same species. In a stimulating article in the recent symposium of the British Society for Parasitology on "Genetic Aspects of Host–Parasite Relationships", Clarke (1976) proposed that the extensive genetic variation in proteins known to occur within outbreeding species is maintained by selection pressures exerted by parasitic infections. Clarke was concerned with biochemical polymorphisms, particularly in enzymes, but also in proteins such as tissue antigens and haemoglobins, which would be likely to alter the physiology of the host and thus alter the balance of the interaction between host and parasite. As yet, however, there are relatively few examples to draw on in order to test such an hypothesis. The best known of these examples are undoubtedly the so-called malaria-dependent-polymorphisms, polymorphisms in man which are maintained by the selection pressures exerted by malarial infection (Livingstone, 1971; Motulsky, 1975) (Table 3).

TABLE 3

Summary of the effects of malaria-dependent polymorphisms upon biological fitness under malarial and non-malarial conditions (Modified from Motulsky, 1975)

	Biological fitness	
	Malarial conditions	*Non-malarial conditions*
Severe α-thalassaemia heterozygote	?Increased	Mild reduction
β-thalassaemia heterozygote	?Increased	Mild reduction
Haemoglobin heterozygote A/S	Increased	Fitness $\simeq$ 1
A/C	Increased	Fitness $\simeq$ 1
A/E	Increased	Fitness $\simeq$ 1
Glucose-6-phosphate dehydrogenase (heterozygote)	Increased	Fitness $\simeq$ 1

The clearest example involves the genetically determined resistance of man to infection with the malarial parasite *Plasmodium falciparum*, a resistance which is known to be associated with the presence of the abnormal haemoglobin S responsible for sickle cell disease. This association was first reported by Allison (1954) and has been reviewed by him in a number of more recent publications (Allison, 1957, 1963, 1964, 1975). The sickle cell condition is produced by a mutation of the gene controlling synthesis of the β-polypeptide chain of haemoglobin with the substitution of a valine for a glutamic acid

residue in each half molecule. In the heterozygote condition less than 50% of the haemoglobin present is type S, the majority is the normal haemoglobin A In homozygotes 80% of the haemoglobin is S. The abnormal haemoglobin has the property of becoming relatively insoluble when in the reduced condition and crystallizes out in the red cells; aggregation of the crystals distorts the cell into the characteristic sickle shape. In homozygotes sickling can take place in the circulation at partial pressures of oxygen that can occur under normal physiological conditions and results in severe haemolytic anaemia and small blood vessel obstruction; indeed, the majority of homozygotes die in childhood. If they survive, homozygote females reproduce with difficulty. Thus there is an intense selection against the sickle gene, yet the frequency of the gene in the heterozygote condition, in which sickling does not occur under normal conditions, is high, frequencies in excess of 20% being common in parts of Africa, Greece, the Middle East and India. Since it is not possible to explain this frequency by postulating a high rate of mutation, it must be due to heterozygote advantage. It was suggested by Allison (1954) that this advantage stemmed from the relative resistance of the heterozygote to infection with *P. falciparum*, the most lethal of the human malarias, and this suggestion has been amply borne out. In young children, before immunity is acquired, heterozygotes carrying the sickle gene show lower parasitaemias than children with normal haemoglobins and have a very low mortality rate from infection (Allison, 1963). The distribution of high frequencies of the sickle gene correlates well with areas where malaria is or has been endemic; eradication of malaria, or movement of populations into non-malarious areas is associated with a reduction in gene frequency (e.g. in American Negroes).

The basis for the increased resistance to *P. falciparum* in sickle gene carriers is unknown at present, although a number of mechanisms have been suggested. Since the resistance appears to be virtually specific to *P. falciparum* the explanation cannot lie in the increased viscosity of haemoglobin S interfering with the uptake and utilization of red cell cytoplasm by the parasite, nor in a lack of the phosphorylating enzymes necessary to make glucose available to the parasite's respiratory pathway (see Garnham, 1966). Similarly there is no evidence to support the suggestion that cells with HbS are less suitable for the development of the parasite (Miller *et al.*, 1956). An alternative proposal, which has some experimental support (Luzzatto *et al.*, 1970), is that, since schizogony in *P. falciparum* occurs in the capillaries of body organs, the lower oxygen tension associated with the presence of the parasite may cause infected cells to sickle, with resultant phagocytosis of the damaged cells. A corollary of the resistance of sickle cells carriers to malarial infection is their relative freedom from a number of syndromes associated with malaria, such as tropical splenomegaly (see Luzzatto, 1972). Interestingly, carriers show a reduced incidence of the nephrotic syndrome associated with *P. malariae* infection (Luzzatto, 1972), a fact which implies that the sickle cell trait confers some resistance to this species as well.

Haemoglobin S is not the only abnormal haemoglobin associated on epidemiological grounds with resistance to *P. falciparum* (Table 3). Haemoglobin C in West Africa, haemoglobin E in South East Asia and

β-thalassaemia in the Near East, India, South East Asia and Africa, have also been implicated (Allison, 1974, 1975; Livingstone, 1971; Motulsky, 1975). In addition, glucose-6-phosphate dehydrogenase deficiency, a sex-linked group of haemoglobin polymorphisms widely distributed in tropical and subtropical regions, is also thought to provide selective advantage to gene carriers in malarious areas as is variation in the ATP level of red cells (Brewer and Powell, 1965; Livingstone, 1971; Luzzatto, 1974; Motulsky, 1975).

It may be that some of these genetically determined abnormal red cell states confer resistance by interfering with the nutritional requirements of the malaria parasite. There is good evidence that alteration of metabolites present in the host may severely interfere with the development of malaria parasites, one of the best known examples being the correlation between deficiency of para-amino-benzoic acid in milk-fed rats and suppression of parasitaemia of *P. berghei* (Hawking, 1953; Jacobs, 1964). Garnham (1966) has suggested that the resistance of West African and American negroes to *P. vivax* (Young *et al.*, 1955; Bray, 1958) may similarly be due to some inherited absence of an essential metabolite, but an alternative explanation has been proposed more recently by Miller *et al.* (1975). They observed that merozoites of *P. knowlesi*, a simian malaria capable of infecting man, were unable in-vitro to penetrate human red cells negative for the Duffy blood group determinants, only 2·2 cells 1000^{-1} becoming infected compared with more than 80 cells 1000^{-1} when the cells carried these determinants. Miller *et al.* (1975) speculated that, since the genotype for the Duffy blood group negative condition is very common among West African and American negroes, it may determine resistance to *P. vivax* infection as well. The basis for the resistance is therefore not nutritional, but the absence on the red cells of receptor sites necessary for the normal attachment and "interiorization" of the merozoite. If this is correct then it is possible that the specificity of this receptor may extend to *P. cynomolgi bastianellii* as well as to *P. knowlesi* and *P. vivax*, since West African negroes are also inherently resistant to this simian malaria, although susceptible to other species (Garnham, 1970). In earlier work McGhee (1950) had shown that ability to invade red cells was a major determinant of host specificity in the avian malaria *P. lophurae*, and it is interesting that subsequently (McGhee and Sullivan, 1971) intraspecific variation in host cells to invasion was also recorded.

B. HELMINTH PARASITES

There appear to be few adequately documented cases where genetically determined susceptibility to helminth infection is known to result from intraspecific variation in innate characteristics, although there are a number of areas in which one can speculate that such variation is likely to occur.

Genetically determined characteristics may influence susceptibility at the initial phases of infection, for example, during the penetration of the body of the host. A number of helminths, for example, schistosome cercariae and nematode larvae, enter the vertebrate host by direct penetration through the

skin. In doing so they must pass through layers of very different physical and biochemical characteristics (Stirewalt, 1963, 1966) including the stratum corneum, basement membrane and the acellular ground substance of the connective tissue. Stirewalt (1956) reported marked differences in success of cercarial penetration through the skin of different host species and also noted intraspecific differences, penetration into hairless mice being less successful than into normal mice, the relative resistance of hairless mice in this respect being due to the nature of the corneal layer. It is interesting in this context to record that as long ago as 1925 Smillie and Augustine suggested that the well-established difference in levels of hookworm infection between negroes and whites was the result of the increased thickness of the negro epidermis, which hindered larval penetration.

It has additionally been shown that the success of penetration depends upon the ease with which the acellular connective tissue constituents of the skin can be altered by larval enzyme secretions (Lewert and Lee, 1957; Lewert and Mandlowitz, 1963). These workers found that as susceptible hosts aged they developed an increased resistance to penetration by *Strongyloides* larvae and by schistosome cercariae and thus showed an increased resistance to infection. This resistance was the result of changes in the ground substance of the dermis (e.g. lower water content, greater polymerization), changes which are presumably under genetic control and which might therefore differ between individuals or between strains of a species. The latter possibility was confirmed by Lewert and Mandlowitz (1963) who showed that a particular strain of mice (C57 Leaden/Heston × A/He), known to age slowly and therefore to retain the skin characteristics of young mice, was as susceptible to penetration by cercariae of *Schistosoma mansoni* when aged 508–1044 days as were CF1 mice at 26 days. Lewert and Mandlowitz suggested that similar mechanisms of resistance may operate wherever helminths penetrate through host tissues.

Since many helminths require a series of appropriate physiological signals from the intestinal environment to initiate and co-ordinate their development in the vertebrate host (Lackie, 1975), it is likely that intraspecific variation in the components of such signals could reduce their effectiveness, adversely affect the development of the parasite and confer resistance upon the host. As far as is known at present, however, the effectiveness of signals is more qualitative than quantitative; their components act over fairly wide ranges and there is little evidence for the necessity of their being present within very precise limits, a situation which would favour the expression of intraspecific variation. Almost the only reference to such a situation is a statement by Weinmann (1970) to the effect that "variations in hatching rates (of cestode eggs) in different hosts undoubtedly complicate assessments of natural resistance based on numbers of worms established from known egg doses". Weinman makes the point that enhanced egg hatching may occur in the "unusually susceptible hosts" which are not uncommonly found in infection experiments, but no evidence to support this statement is given.

Cestodes may prove to be the most rewarding helminths in which to look for examples of intraspecific variation based on innate host characteristics, since they are most intimately affected by the physiology of the host. Cestodes

are known to be markedly influenced by the constituents of host bile, which act not only as components of developmental signals, but as factors regulating activity and migration and indeed are capable of directly damaging the tegument (e.g. lysis of *Echinococcus granulosus* protoscoleces by herbivore bile). Although bile from different species has been analysed in some detail (Smyth and Haslewood, 1963), there is little information about the extent of intraspecific variation in composition which could possibly influence susceptibility to infection. Smyth and Haslewood (1963) did find that bile taken from dogs of different breeds had varying effects upon *E. granulosus* when tested *in vitro*, but pointed out that many other factors, including the state of health of the dogs, could have brought about this variation.

C. ARTHROPOD PARASITES

Parasitic arthropods, almost without exception, need to penetrate the epidermal layers of the host in order to feed and it is therefore entirely possible that intraspecific variation in resistance to infection may arise from factors similar to those discussed in relation to helminth invasion. There appears, however, to be very little information available, although differences in coat characteristics have been suggested as a minor factor in resistance of certain breeds of cattle to tick infestation (Francis and Little, 1964). There is indeed overwhelming evidence that, where resistance is due to the inability of the arthropod to feed adequately, the factors responsible arise from immunologically-mediated changes in the skin. This aspect will therefore be treated more fully in Section VI.

The blood-feeding habits of ectoparasitic arthropods provide a further source of intraspecific variation, in that the parasite is intimately exposed to the biochemical environments of the tissue fluids and blood and may be adversely affected by them. One example which is relevant comes from the work of Hall and Gross (1975) who found that smaller populations of the northern fowl mite, *Ornithonyssus sylviarum*, developed on cockerels selected for high plasma corticosterone response to stress than on cockerels from a low corticosterone line. The underlying relationship between high corticosterone levels and relative resistance to infection was not investigated, but an effect upon mite reproduction was one possibility raised. Since the effect of corticosterone is normally to depress immune responses made against metazoan parasites it would appear that resistance did in this case reflect innate characteristics of the host. Although their experiments were designed to investigate the interactions of stress and corticosterone response in the host upon the parasite, Hall and Gross found that the inherited levels of corticosterone response were the major influence and their effect on the parasite was not affected by variations in stress experienced by the host. This raises the possibility that the original selection for corticosterone response may have been accompanied by selection for some other characteristic which was ultimately responsible for the observed effect on the mite population.

V. Acquired Host Characteristics and Resistance to Infection

Experience of infection brings about a variety of changes in the environment provided for the parasite by the host. Some of the changes may lead to a greater susceptibility to infection, but when the host is susceptible initially it is more likely that the changes will render the host less suitable for the continued survival of the parasite or may reduce susceptibility to reinfection, in other words, the changes will confer resistance. Resistance can be conferred by changes in the host which owe nothing to the immune system, for example, the thickening of epidermal structures after helminth penetration or reduction in the availability of cells suitable for intracellular protozoans, but in the majority of cases it is probable that resistance stems directly or indirectly from the host's capacity to recognize the parasite as "not-self" and to mount an immune response against it.

In relatively few of the reported cases of intraspecific variation is conclusive evidence given that variation was the consequence of immunological activity against the parasite concerned. Many reports attempt no analysis of the underlying mechanisms beyond attribution to an unspecified "resistance" and a number were made before there was any understanding of the nature of immune responses to parasites. Nevertheless, where intraspecific variation is manifested as a reduction in parasite growth or survival after a period of normal establishment and development, it would seem reasonable to assume that immunologically-based anti-parasite activity is implicated. This assumption is strengthened by the similarities which exist between examples of variation known to involve differential responsiveness and those where no mechanism has been proposed. In addition, there are many analogies which can be drawn between intraspecific variation to parasitic infections and that to viral and bacterial infections, in which the importance of immunological mechanisms has been amply demonstrated.

It is important at this point to emphasize the distinction between the use of the term resistance in the sense considered above and the use of the term to describe the capacity of an animal to withstand the pathogenic consequences of infection. In a number of instances, intraspecific variation in susceptibility has been assessed by the survival or death of individuals after infection. Clearly resistance in this sense may imply the operation of immune responses which limit the activity of the parasite and thus reduce pathology, but this is not necessarily true in all cases. Survival in the face of parasitic infection can reflect a tolerance to physiological disturbances, for example, reduced haemotocrit, which is essentially an innate and not an acquired characteristic. However, it is often not possible to distinguish between the two situations and for convenience all cases of variation based on survival data will be considered in Section VI.

To provide a background for discussion of the examples of intraspecific variation which will be considered in Section VI it will perhaps be useful to itemize briefly the major immunological mechanisms thought to be involved

in protective immunity against parasites and to review in some detail what is currently known of the genetic control and genetically-based variation of such mechanisms.

A. MECHANISMS OF PROTECTIVE IMMUNITY

There have been a number of excellent reviews of this subject in recent years and for detailed information the reader is referred to them (Taylor, 1968; Taliaferro and Stauber, 1969; Jackson *et al.*, 1970; Jarrett and Urquhart 1971; Soulsby, 1972; Ogilvie and Jones, 1973; Mauel and Behin, 1974; Ogilvie, 1974; Cohen and Sadun, 1976).

1. *Protozoa*

Anti-parasite antibodies and mononuclear phagocytic cells are major components of protective immune responses to both intracellular and extracellular protozoans. Antibody (IgM, IgG) may act directly, for example by causing agglutination of organisms, or may act in conjunction with components of the complement system with resultant opsonization or lysis of the parasite. Agglutinated parasites are taken up by a variety of phagocytic cells; opsonization facilitates ingestion by macrophages. Macrophages may also be rendered cytotoxic in the presence of anti-parasite antibodies. In all cases so far studied, protective antibody production appears to be thymus helper-cell dependent. Macrophages can be "activated" against protozoans, i.e. their powers of phagocytosis and intracellular lysis enhanced, by the activity of thymus-dependent lymphocytes responding to the parasite's antigens. More direct parasite-lymphocyte interactions have been proposed but experimental support for their occurrence is limited at present.

2. *Digenea*

Analysis of protective immune responses is essentially restricted to two species, *Schistosoma mansoni* and *Fasciola hepatica.* In both, immunity is thymus-dependent and can involve the action of anti-parasite antibodies. The role of thymus-derived lymphocytes other than in helper-cell activity is uncertain. In systems *in vitro* eosinophils and neutrophils have been shown to play a part in schistosome destruction. *In vivo*, schistosome infections are associated with marked delayed hypersensitivity responses.

3. *Cestoda*

Antibodies (IgG) are known to confer protection *in vivo* against larval stages of cestodes and the importance of complement has recently been demonstrated. Inflammatory responses are also involved in the destruction of larvae. Immunity against adult worms in the intestine is known to be thymus-dependent and some evidence is available for a protective role of antibodies.

4. *Nematoda*

Analysis of protective immunity has been confined largely to intestinal species. In all cases immunity is thymus-dependent. Protective activity of antibody (IgG) is well established in some systems; in all, the additional involvement of T-cell activity is suspected. Inflammatory responses involving neutrophils, eosinophils or basophils are thought to play an important role in bringing about ultimate expulsion. A number of species stimulate high levels of IgE antibody and immediate hypersensitivity has been proposed as an element in expulsion. Immunity to tissue-penetrating nematodes is less well studied, though there is evidence of the involvement of anti-worm antibodies and inflammatory responses.

5. *Arthropods (ectoparasites)*

Protective immunity is largely concerned with prevention of feeding and it is known that in some cases antibodies directed against salivary secretions are involved. Hypersensitivity reactions are common, both immediate and delayed, and may play an important role in producing changes in the skin which are deleterious to the parasite.

B. GENETIC CONTROL OF THE IMMUNE RESPONSE

Immune responses, in particular those made against parasitic organisms, are complex sequences of events involving a wide variety of the defence mechanisms of the body. All responses, however complex their development and expression, involve as an essential, initial step, the recognition of antigenic determinants by specific receptor molecules carried on the surface of lymphocytes. Specific antigen recognition induces proliferation of the cells bearing the appropriate receptors and frequently leads to increased synthesis and release of receptors as antibody. The immunologically specific phase of an immune response may then be followed and amplified by interactions with a number of non-specific components. For example, interaction of antigen with thymus-derived lymphocytes leads to the production and release of factors (lymphokines) which have a wide range of biological activities, such as modulation of lymphocyte function, attraction and activation of phagocytic cells, stimulation of stem cell production and so on. Antibody may alter the behaviour of lymphocytes and phagocytic cells towards the source of the antigen concerned and antigen–antibody combination leads to a multiplicity of events, of which those of complement activation and interaction with amine-releasing cells are of particular significance. The genetic control of immune responses can therefore operate at a variety of levels, determining in quantitative and qualitative fashion not only the basic processes of specific recognition but also the consequential events which recognition may entail (Fig. 3). A comprehensive account of how such genetic controls operate is beyond the scope of this article. Immunogenetics is one of the most rapidly advancing areas of modern immunological research and is documented by an extensive literature, recent

reviews of which have been given by Herzenberg *et al.* (1968), McDevitt and Benacerraf (1969), Mozes and Shearer (1972), McDevitt and Landy (1972), Benacerraf and Dorf (1974), Gasser and Silvers (1974), Hildemann (1974), Sercarz *et al.* (1974), Benacerraf and Katz (1975) and Weigert *et al.* (1975); what follows is intentionally limited to those aspects of immunogenetics which may be relevant to the theme of intraspecific variation in immunity to parasitic organisms.

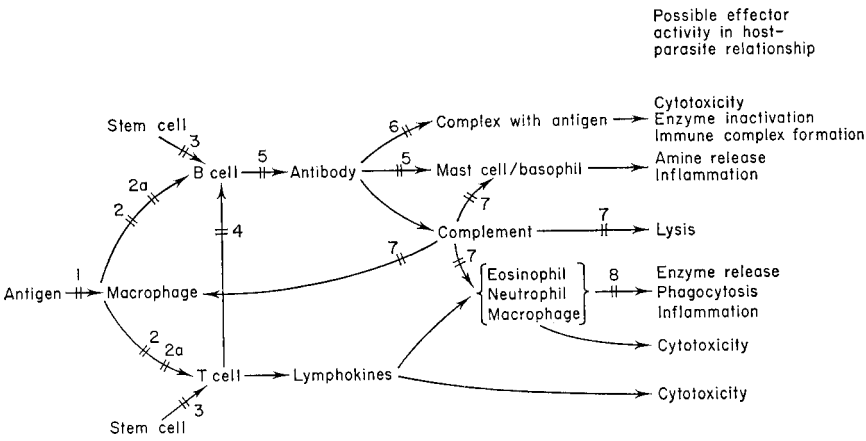

FIG. 3. Diagram showing points at which genetic control may be exerted during the development and expression of anti-parasite immune responses.
1. Antigen handling by macrophage
2. Antigen presentation by macrophage
2a. Antigen recognition by lymphocyte
3. Stem cell deficiency
4. T-B cell interaction (helper/suppressor function)
5. Control of antibody production (class/level/specificity)
6. Antibody affinity
7. Defects in complement components affecting lysis, chemotaxis, opsonization
8. Defective population or activity

The ability of the vertebrate immunological mechanism to recognize and respond to an enormous variety of naturally occurring and synthetic antigens is one of its most remarkable characteristics. It has been known for some time, in human medicine, that recognition and responsiveness can be grossly defective as a result of congenital abnormalities in various components of the immune system (Rosen, 1972; Soothill, 1974). Deficiencies may be evident either in antibody-mediated responses, for example, Burton's disease (infantile X-linked hypogammaglobulinaemia) or in cell-mediated responses, such as the di George syndrome (thymic aplasia); combined deficiencies also occur. Unless corrected, these defects result in chronic infection and early mortality and obviously would not contribute extensively to intraspecific variation in animal populations, although one such defect, thymic aplasia in the nude mouse, is exploited as a laboratory model of selective T-cell deficiency. What

is more relevant is the occurrence of restricted, genetically determined defects in immunological recognition and response, defects which do not markedly reduce the survival of the individual under normal circumstances. Such defects can be considered under three headings (1) defects in responsiveness to specific antigens, (2) defects in general responsiveness to antigens, (3) defects in immunologically non-specific effector mechanisms.

1. *Defects in responsiveness to specific antigens—immune response genes*

Although it has been recognized for some time that individual members or inbred lines of a species may be unable to respond to antigens to which other members of the species are fully responsive, it is only relatively recently that the genetic basis of such phenomena has been unravelled. Essentially, specific defects of this type are due to the absence of genes coding for the appropriate immunoglobulin combining site or of genes which control the ability of lymphocytes to respond normally to the antigen concerned.

TABLE 4

Antigens to which the immune response of mice is known to be under H_2-linked Ir *gene control*
(Modified from Benacerraf and Katz, 1975)

Synthetic polypetides	Linear copolymers of the L-amino acids, glutamic acid, lysine, alanine, tyrosine, proline, phenylalanine.
	Branched copolymers of L-amino acids.
Mouse alloantigens	IgA, IgG } Balb/c myelomas
	H-Y ♂histocompatibility antigen
	Thyl. 1 Thymocyte antigen
	Ea 1 Blood group antigen
Foreign antigens	Ragweed extract
	Staphylococcal nuclease
	Leukaemia associated transplantation antigen
	Ovomucoid
	Ovalbumin
	Bovine gammaglobulin
	Lactic dehydrogenase (sub unit B)

The work of Benacerraf and McDevitt, together with that of their collaborators (McDevitt and Benacerraf, 1969) established that the ability of individuals or inbred lines of mice and guinea-pigs to respond to a variety of antigens was under the control of autosomal dominant genes, to which the term immune response (*Ir*) genes was originally applied. A variety of antigens has been studied in this context (see Table 4) but the most productive work has utilized synthetic polymers of a small number of amino acids, providing antigens of limited heterogeneity and specificity. McDevitt and Chinitz (1969)

showed that, in the mouse, *Ir* genes controlling responses to certain synthetic polypeptides were linked to the genes coding for the major histocompatibility antigens and it is now known that many *Ir* genes are in fact located within the H_2 complex (Benacerraf and McDevitt, 1972; Benacerraf and Katz, 1975; Shreffler and Cheller, 1975). The existence of histocompatibility (H) linked *Ir* genes has also been demonstrated in guinea-pigs (Ellman *et al.*, 1970), rats (Armerding *et al.*, 1974) and primates (Dorf *et al.*, 1974) and there is strong evidence for their existence in man (Levine *et al.*, 1972; Buckley *et al.*, 1973).

Histocompatibility-linked *Ir* genes control both antibody and cell-mediated responses and their activity can be detected by measuring antibody titres, plaque-forming cell responses and delayed hypersensitivity reactions of the classical and cutaneous basophil types. There is good evidence that *Ir* genes control responsiveness at the level of the lymphocyte and are not expressed on macrophages, but there is controversy whether gene expression occurs on T-cells, B-cells or both. For a number of H-linked *Ir* genes there is good evidence of expression on T-cells. For example, animals which are genetically incapable of responding to a particular antigen can respond if the antigen is presented on an immunogenic carrier molecule (Green *et al.*, 1966; Dunham *et al.*, 1972; Debre *et al.*, 1975a). Since carrier recognition is a known T-cell function, the absence of response implies a T-cell defect. It has also been observed that non-responders possess as many B-cells capable of binding the specific antigen as do responders (Dunham *et al.*, 1972). In guinea-pigs, animals lacking *Ir* genes are true non-responders, but in mice non-responders to certain antigens are more accurately described as low responders since they are capable of some response. It was shown by Mitchell *et al.* (1972) that mice which were low responders to the synthetic polypeptide (T,G)-A--L (tyrosine, glutamic acid, alanine, lysine) could produce IgM antibody on challenge, but were unable to switch to IgG production in the normal way, i.e. they showed a defect in T-cell function. More recently, evidence has accumulated that the expression of *Ir* genes may be specifically on T-helper cells, since it has been shown that non-responder mice do generate suppressor T-cells on stimulation with antigen (Kapp *et al.*, 1974; Debre *et al.*, 1975a). Debre *et al.* (1975b) have in fact suggested a class of immune-suppression genes controlling this suppressor activity.

Despite this convincing evidence of T-cell involvement in *Ir* gene expression there are other experimental data to support the view that expression can be on B-cells and certainly there are now a number of *Ir* genes known which determine responsiveness to T-independent antigens (Amsbaugh *et al.*, 1972; Watson and Riblett, 1974, 1975) although this in itself is not conclusive proof of B-cell involvement. Much of the data has come from the work of Mozes and Shearer (Mozes and Shearer, 1972; Mozes, 1974) who have shown B-cell expression in both non H_2-linked and H_2-linked *Ir* genes in mice. It is apparent that the site of the defect may well depend upon the antigen and the strain of animal concerned.

The nature of the *Ir* gene products and their relation to histocompatibility antigens is still unclear, as is the role of the products in determining lympho-

cyte co-operative responses (for reviews of current knowledge see Katz and Benacerraf (1975, 1976) and Blanden *et al.* (1976)).

The association of *Ir* genes with the major histocompatibility complex means that for certain antigens the status of an animal as responder or non-responder can be predicted from knowledge of its histocompatibility type. However, there are many reported correlations of histocompatibility type with immune responsiveness or disease susceptibility in which the operation of *Ir* genes has not been demonstrated. *A priori* it is likely that many of these examples will be found to involve *Ir* genes but other explanations are possible. The histocompatibility loci control a variety of tissue antigens, some recognizable serologically, others recognizable only by the responses of allogeneic lymphocytes in in-vitro tests such as the mixed lymphocyte reaction and cell-mediated lympholysis. An interesting suggestion is that failure to respond to certain antigens may therefore arise if the test antigen shares determinants with antigens present in the body of the animal and to which that animal is tolerant. Such a possibility has been demonstrated experimentally for host responses to tumour antigens, using an in-vitro T-cell mediated cytotoxicity system (Vachek and Kölsch, 1974) and is probably also relevant to infection by pathogenic organisms where the possession of such antigens would facilitate invasion and survival in the bodies of host animals. This idea was discussed in a parasitological context by Damian (1964), who termed the phenomenon "molecular mimicry", and its significance in the context of polymorphism of histocompatibility loci was considered by Snell (1968). Snell discussed the possible relationship between polymorphism and susceptibility to pathogens which shared histocompatibility antigens with the host and came to the conclusion that polymorphism could be maintained by the selection pressure exerted by such pathogens, the variety of alleles being "like different locks on different doors, which increase the difficulty of the burglar in gaining entrance".

Whatever the underlying causes, the association of histocompatibility type with disease is of obvious significance in medicine and is being intensively studied (Morris, 1974; Vladutiu and Rose, 1974; Transplantation Reviews, 1975 vol. 22). The diseases showing HL-A association in man include a number with viral and bacterial aetiology, neoplastic diseases, autoimmune diseases, allergies and other immune disorders and some of unknown aetiology. It appears that, of the major parasitic diseases of man, HL-A association has been recorded only in the case of malaria (Cepellini, 1972).

Ir genes which are not linked to the major histocompatibility complex have also been identified in recent years. In some cases the gene showed sex-linkage (Amsbaugh *et al.*, 1972; Mozes and Fuchs, 1974), in others the gene was autosomal (Gasser, 1969; Schmitt-Verhulst *et al.*, 1974). The gene studied by Gasser, which controls responsiveness to the Ea-1 blood group system in mice, differs from the majority of *Ir* genes in that responsiveness is inherited as a recessive trait. An important group of non-histocompatibility-linked *Ir* genes are the immunoglobulin–allotype-linked genes which are thought to be associated with, or identical to, the germ-line genes coding for the variable regions of immunoglobulin molecules and thus control the ability of an

animal to recognize antigenic determinants at a basic level. *Ir* genes of this type have been detected only by using antigens which elicit antibodies of restricted heterogeneity, such as α-l, 3-Dextran (Blomberg *et al.*, 1972) and pneumococcal C carbohydrate (Sher and Cohn, 1972).

The experimental demonstration of *Ir* genes and analysis of their activity have been carried out primarily using synthetic antigens of limited heterogeneity, but more complex multideterminant antigens have been used (Table 4). In the latter case it has been found necessary to use the antigens in limiting immunizing doses, so that only the most immunogenic determinants of the antigen are recognized. Under these circumstances *Ir* gene activity can be detected. Since *Ir* gene activity is unlikely to be limited in nature, it is possible that responsiveness to many complex immunogens is genetically controlled, but such control will not be recognized because of the individual animal's capacity to respond to some, at least, of the antigenic determinants present (Lozner *et al.*, 1974). However, there are a number of cases where disease susceptibility involving, presumably, response to complex immunogens, is thought to be determined by *Ir* genes, for example leukaemogenic and other virus infections (Lilly and Pincus, 1972; Oldstone *et al.*, 1973; Darnell *et al.*, 1974; McDevitt *et al.*, 1974), bacterial infections (Plant and Glynn, 1974a, 1976), and autoimmune conditions (Gasser *et al.*, 1973; McFarlin *et al.*, 1975; Silver and Lane, 1975).

2. *Defects in general responsiveness to antigens*

The qualitative defects in response discussed above were correlated with control exerted by single *Ir* genes. Where control of responsiveness is polygenic, which perhaps covers a majority of cases, defects are essentially quantitative, although the extremes of high and low responsiveness may suggest a qualitative difference. One of the most thoroughly studied examples in this category is the antibody response of strains of mice to sheep red blood cells (SRBC). Variability in response has been known for some time (Davidson and Stern, 1955) but has been most fully investigated by Biozzi and his co-workers (reviewed by Biozzi *et al.*, 1971; Stiffel *et al.*, 1974). Beginning with a number of random-bred albino mice, separated on the basis of anti-SRBC response into high and low responders, Biozzi carried out a two-way selection experiment, mating high responders with high responders and low responders with low responders. For the first six generations the progeny were selected on the basis of their anti-SRBC response, but subsequently immunization and selection were carried out using SRBC and pigeon blood cells in alternate generations to prevent any interference with selection by maternally-derived antibody. Although there was no cross reactivity between the two species of erythrocytes, high response to SRBC was correlated with high response to pigeon cells and similarly with low response. After nine generations the selection lines differed in antibody titre some thirty-fold and the difference increased up until the twentieth generation by which time the lines were considered homozygous for the character selected (Fig. 4). It was found that the lines differed in response not only to SRBC and pigeon cells but also to the O and H antigens of *Salmonella typhi*, to hen ovalbumin,

pneumococcal polysaccharide SIII, *Limulus* haemocyanin, bovine serum albumin, DNP-hapten and T_4 bacteriophage. Selection had been for genes concerned with regulation of immunoglobulin synthesis in general and had not been antigen specific, although Howard *et al.* (1974) subsequently found that the two lines did not differ in response to levan and dextran B1355. Selection also had had little effect upon T-cell-mediated responses. The phenotype "high responsiveness" was found to be dominant and was expressed unaltered in the F_1 generation. Analysis of the data gained from this

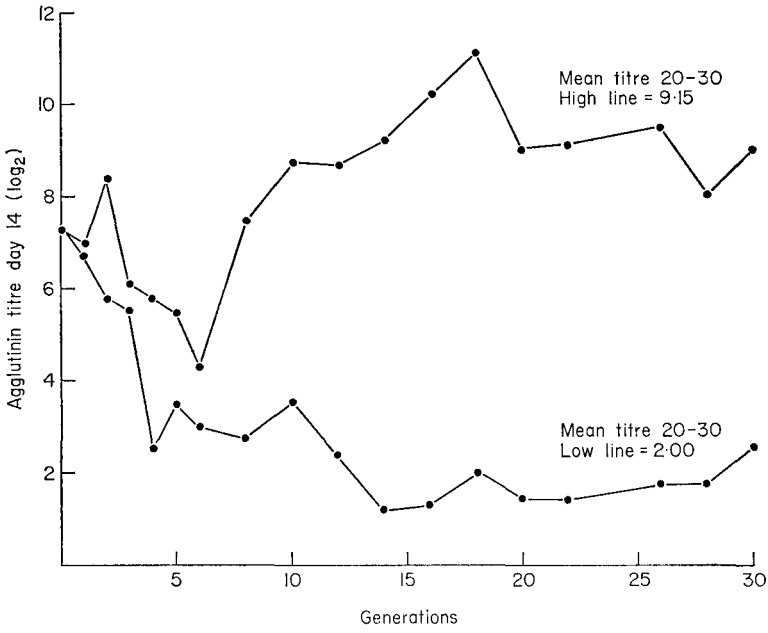

FIG. 4. Separation by selective breeding of lines of mice showing high and low anti-SRBC agglutinin response after injection of 5×10^8 SRBC. (Redrawn from Stiffel *et al.*, 1974.)

and a subsequent selection experiment suggested that approximately ten different loci were involved in the control of antibody production. It has been suggested (Stiffel *et al.*, 1974) that the loci concerned include histocompatibility-linked and allotype-linked *Ir* genes. In addition, there is evidence (Howard *et al.*, 1974; Wiener and Banduri, 1974) that genetic control of macrophage function is also involved; the macrophages of the low responders being more active and degrading antigen more efficiently than those of the high responders.

Mice of the Biozzi high and low responder lines have been used to investigate the immune responses stimulated during infection with *Trichinella spiralis* (Perrudet-Badoux *et al.*, 1975) and *Trypanosoma cruzi* (Kierszenbaum and Howard, 1976). The results obtained with these infections will be discussed in Section VI.

The polygenic system studied by Biozzi exerted control on the overall levels of antibody production. After immunization with SRBC, high responder mice had total serum immunoglobulin concentrations which were six times greater than low responder mice and analysis showed that the levels of IgM IgG and IgA were all increased (Biozzi *et al.*, 1970). When the mice were challenged with hen ovalbumin, the high responder line produced markedly higher levels of IgE and IgG skin-sensitizing antibodies. In addition to this example of a generalized control upon the class of immunoglobulin produced in response to antigenic stimulation, there are a number of examples known in which genetic factors determine whether or not a specific immunoglobulin class is produced. Silver *et al.* (1972) found that C57Bl/10 mice made a predominantly IgM response to repeated injections of SRBC and failed, unlike other strains, to switch to IgG production. It was considered that here too polygenic control was involved. In man there is evidence for X-linked genes which determine in a quantitative manner the amounts of IgM present in the serum (Grunbacher, 1972). Genetically determined defects in the ability to make IgA antibody are also known in man (Heremans, 1974). Because of a compensatory increase in mucosal IgM levels, this deficiency is compatible with a normal life and does not result in chronic infection of mucosal surfaces.

A great deal of attention has been concentrated upon the genetic control of reaginic (IgE) antibody production in man and animals because of its clinical importance in allergic conditions. Since parasitic, and particularly helminth, infections are potent stimulators of reaginic antibody production (Ogilvie and Jones, 1969), this area of genetic control is of particular significance to this review. It has been known for some time in man that there is familial predisposition to allergy and strong circumstantial evidence for a genetic basis for the atopic condition (Marsh, 1975). From a study of HL-A-associated ragweed allergy Levine *et al.* (1972) suggested that the association involved both an HL-A linked *Ir* gene determining the specificity of response to allergens and a second, unlinked gene controlling the production of reaginic antibody. Marsh *et al.* (1974) concluded that high IgE production was inherited as a simple Mendelian recessive trait, a mode of inheritance also implicated by Schwartzman *et al.* (1971) for atopy in dogs. For some time experimental study of the control of IgE production was hindered by the lack of suitable models; ironically one of the reasons for this lack has turned out to be intraspecific variation in the ability to mount IgE responses. Munoz and Bergman (1968) found a marked variation in the responsiveness of mouse strains to the histamine-sensitizing factor (HSF) of *Bordetella pertussis* vaccine. Mice producing a good response — high IgE levels—died rapidly from small amounts of injected histamine whereas poor responders survived larger amounts. Wardlaw (1970) concluded that the inheritance of responsiveness to HSF involved a single dominant autosomal gene, but Ovary and Caiazza (1975) considered that polygenic control was involved.

Intraspecific variation in IgE production after stimulation with controlled antigen–adjuvant preparations has been recorded by Revoltella and Ovary (1969) and Levine and Vaz (1970a). The latter showed that repeated injection

of small (0·1 μg) amounts of antigen adsorbed on aluminium hydroxide gel evoked high IgE titres in four of eight inbred mouse strains, but had no effect in the remainder; injection of a single large dose of antigen–adjuvant induced responses in all strains. In addition to the IgE response to small amounts of antigens, the responder strains produced IgG antibody (detected by haemagglutination) whereas, again, the non-responders produced none. Levine and Vaz concluded that the difference in responsiveness reflected a capacity to respond to repeated small amounts of antigen rather than a specific IgE response, although the latter response predominated. In subsequent reports (Levine and Vaz, 1970b; Vaz *et al.*, 1971) it was proposed that IgE responsiveness to low doses of antigen involved H_2-linked genes determining specific recognition of the antigen and a second genetic control of reaginic antibody production. In the experimental system used by Levine and Vaz (hapten-carrier–aluminium hydroxide adjuvant) responder mice produced IgG_1 and IgE skin sensitizing antibodies at low dose levels; at high dose levels some non-responders produced both IgG_1 and IgE, others produced only IgG_1, showing lack of correlation between genetic control of the two immunoglobulin types.

One of the mouse strains used in this work, SJL, has been extensively studied by subsequent workers. Gollapudi and Kind (1975) suggested that the non H_2-linked control of reaginic production was expressed at the level of T-cells since they found that incorporation of Concanavalin A (a T-cell mitogen) with antigen could boost IgE levels, presumably by T-cell stimulation of B-cell response. Watanabe *et al.* (1976) have shown that IgE production in SJL is actively suppressed by non-specific suppressor T-cells. SJL mice are also known to be poor reagin responders when infected with *T. spiralis* (see Section VIB).

Not only has it been shown that inbred mice vary in their ability to produce IgE responses on antigenic stimulation but it has also been demonstrated that there is variability in their susceptibility to passive cutaneous anaphylaxis (de Souza *et al.*, 1974) by injected IgE. Thus the choice of mouse strain for such tests may be critical. de Souza *et al.* also found variability in susceptibility to PCA by IgG_1, but there was no correlation with susceptibility to IgE PCA.

Macrophages play a vital role in the initiation of many immune responses (Unanue, 1972) and genetically determined defects in macrophage function exert a marked effect upon general responsiveness. There is evidence from studies on mice carrying *Ir* genes for responsiveness to synthetic polypeptides (Gallily and Eliahu, 1974) and SRBC (Wiener and Bandieri, 1974) that the ability of macrophages to handle and process antigen differs markedly in high and low responder animals, although opposite conclusions were reached by these workers. The former found that high responder macrophages were most efficient, the latter low responders. In man, defective macrophage processing of antigen is thought to be a contributory factor in the X-linked recessive immunodeficiency known as the Wiscott-Aldrich syndrome (Blaese *et al.*, 1968).

Variation in macrophage activity has also been implicated in the genetic control of antibody affinity (ability to combine with antigenic determinants). Inbred strains of mice are known to differ in the affinity of the antibodies they

produce to test antigens (Petty *et al.*, 1972) and selective breeding for the characteristics of high and low antibody affinity has been successfully achieved in an outbred stock (Katz and Steward, 1975). Passwell *et al.* (1974) have shown that mice producing low affinity antibody have poorer macrophage function and Morgan and Soothill (1975) have suggested that the relationship arises from selection by poorly processed antigen of B-cells bearing immunoglobulin receptors of low affinity. As Soothill and his co-workers have pointed out (Alpers *et al.*, 1972; Petty *et al.*, 1972) low affinity antibody production and defective clearance of antigen–antibody complexes may contribute to the development of immunopathological conditions such as immune complex disease. Soothill *et al.* (1975) have discussed this aspect of genetically determined immunodeficiency with specific reference to pathological consequences of parasitic infection, for example malarial nephrotic syndrome.

Defective macrophage function has also been shown to influence the induction of tolerance to certain protein antigens in mice. Lukic *et al.* (1975) found that the susceptibility of DBA/2 mice to induction of tolerance by ultracentrifuged bovine gamma globulin stemmed from the inability of their macrophages to process the small immunogenic component present in the antigen. The macrophages of Balb/c mice, which were resistant to tolerance induction, were able to do this.

A final aspect of genetically determined influences upon general responsiveness to antigens, and one that may have some relevance to parasitic infections at the population level, is that of the decline of immunological competence with aging. Humoral and cell-mediated responses tend to decrease with age and there may also be decline in suppressor cell activity (Good and Yunis, 1974; Nordin and Makinodan, 1974; Stutman, 1974). Inbred strains of mice are known to age immunologically at different rates and this may contribute to intraspecific variation in response when mice of comparable age are challenged.

3. *Defects in immunologically non-specific effector mechanisms*

In addition to their role in initiation of immune responses macrophages are a major component of effector mechanisms, being capable of both phagocytic and cytotoxic activity in response to specific stimuli from antibodies and lymphokines. Genetically determined defects in these activities, though qualitatively similar to those discussed in the previous section, are reflected in impaired ability to deal with invading pathogenic organisms. Bang and Warwick (1960) found that adult Princetown strain mice were susceptible to, and died from, infection with mouse hepatitis virus, whereas C3H mice were resistant, resistance and susceptibility being controlled by a single dominant genetic factor. Tests *in vitro* showed that the macrophages from Princetown mice supported virus replication and were destroyed, but those from C3H mice prevented virus multiplication and cleared the infection. Similar observations with other viruses were made by Goodman and Koprowski (1962) and it is apparent from this work that the resistance of macrophages is specific, in that cells from a particular mouse strain may be resistant to one virus and simultaneously susceptible to another.

A comparable situation has been described by Medina *et al.* (1975) who found that C57Bl/6J mice were susceptible to *Salmonella typhimurium*, but inherently resistant to *Listeria monocytogenes*, whereas A/J mice showed the reverse relationship. From studies using the specific bacterial vaccines, BCG and the non-specific polynucleotide stimulant poly (I:C) it was concluded that there was a specific impairment of macrophage microbiocidal activity, since even non-specific activation failed to induce resistance in macrophages to the bacterium for which the mouse strain was genetically susceptible.

Deficient non-specific effector mechanisms may also involve defects in the polymorphonuclear leucocyte (PMN) population, although to date examples are known only from man (Valdimarsson, 1975). Two representative defects can be mentioned, chronic granulomatous disease, an X-linked recessive condition in which phagocytic cells fail to produce hydrogen peroxide, a major factor in the phagocytic killing of pathogens, and hereditary neutropenias in which the total number of PMN is reduced. One variety of neutropenia, the lazy-leucocyte syndrome, involves both qualitative and quantitative defects in that the PMN also show decreased random mobility and chemotactic responses.

An important area of genetically determined variation in non-specific defence mechanisms involves defects or polymorphisms in components of the complement system. Activation of complement by antibody–antigen interactions leads to a multiplicity of events of significance in the immune response, ranging from influences on basic processes of antigen processing and recognition to effector activity directed against target cells or organisms. The consequences arising from defects in complement depend upon the component involved (Alper and Rosen, 1971, 1974). Deficiencies of initial components (C_{142}) may result in defective antigen processing, deficiency in C_3 results in severe immunodeficiency disease because of failure of lysis and opsonization of pathogens, deficiency at the levels of C_5, C_6 or C_7 may result in impairment of phagocytic or chemotactic activity and interference with lysis. Deficiencies of C_1, C_2, C_3, C_4, C_5, C_6 and C_7 are known in man; C_4 in guinea-pigs; C_5 in mice and C_6 in rabbits and hamsters. Some complement deficiencies are known to be histocompatibility-linked in man and in the mouse, and in the latter there is evidence for relatively simple genetic control (Rosenberg and Tachibana, 1969).

4. *Genetic variation in immune responsiveness and the host–parasite relationship*

The possible involvement of immunological mechanisms in genetically determined variability in response, and particularly in unresponsiveness, to parasite infection has been discussed in general terms by Sprent (1959, 1969), Damian (1962, 1964) and by Dineen (1963a,b). In 1959 Sprent suggested that an established host–parasite relationship may evolve towards a state of diminished immunological interaction—a process he termed "adaptation tolerance"—as a result of the antigens of the parasite changing to resemble those of the host and of the selective obliteration of parasite antigen

combining sites in the host. In his later article Sprent (1969) amplified these ideas in the light of current immunological knowledge and in effect concluded that unresponsiveness to parasite antigens may arise because the antigens resembled those of the host or because there were genetically determined specific defects in recognition and response at the level of the macrophage and lymphocyte.

Damian (1962) coined the term "eclipsed antigens" to describe parasite antigens which elicited minimal response in the host because of their similarity to host antigens. In 1964 he discussed the wider aspects of "molecular mimicry" in parasites and suggested that parasites may synthesize host-antigens as an evolutionary stratagem to counter the effectiveness of immune responses. Damian's views were supported by reference to examples, primarily from helminths, of the occurrence of host antigens in parasite tissues, but the phenomenon is known to occur in many disease organisms (Jenkin, 1963).

The ideas discussed by Sprent and Damian were put forward before there was any great understanding of the genetic control of immune responses and it is interesting to see how well Sprent's concept of genetically determined defects in recognition and response agrees with present knowledge of *Ir* gene function. Similarly, Sprent's hypothesis of unresponsiveness due to antigenic similarity and Damian's ideas on molecular mimicry agree well with more recent suggestions of histocompatibility antigen sharing between host and pathogen. What these workers did not emphasize, nor indeed was the information available to them, was the range of genetically controlled variation in immunological capacity potentially present in host populations, allowing the possibility of parasite survival in an otherwise unsuitable species and the converse, the reservoir of genetically determined resistance in species that are normally suitable hosts. The evolutionary implication of such variation for both host and parasite is obviously of great potential significance and will be referred to again in a later section.

VI. Vertebrate Host—Parasite Relationships: Genetic Control Mediated via Acquired Host Characteristics

A. PROTOZOAN PARASITES

Although there is no reason to believe that intraspecific variation in host response occurs less frequently in infections with protozoan than with helminth or arthropod parasites (see Table 5), it is possible that it may be less readily detected. The capacity of Protozoa to reproduce within the host may result in differences in susceptibility between individual hosts being masked by selection and adaptation in the reproducing parasite population. Adaptation in this sense may not necessarily imply the occurrence of antigenic variation, although this is an obvious possibility and one that further complicates the interaction between the genotypes of the host and parasite. Evidence for the

TABLE 5

Vertebrate host–parasite relationships in which genetically determined intraspecific variation in susceptibility and resistance has been demonstrated

Parasite	*Host*	*Reference*
PROTOZOA		
Trypanosoma congolense	Cattle	Chandler, 1952, 1958; Desowitz, 1959
T. vivax	Cattle	Roberts and Gray, 1973
T. brucei	Mouse	Herbert and Lumsden, 1967; Clarkson, 1976
T. cruzi	Mouse	Goble, 1970; Marr and Pike, 1967; Kierszenbaum and Howard, 1976
Plasmodium falciparum	Man	See text
P. vivax	Man	Young *et al.*, 1955; Bray, 1958
P. cynomolgi	Man	Garnham, 1970
P. bastianelli	Man	Garnham, 1970
P. berghei	Mouse	See text
P. vinckei	Rat	Zuckerman, 1968
Leishmania donovani	Mouse	Bradley, 1974; Smrkovski *et al.*, 1974
Eimeria tenella	Fowl	See text and Table 7
E. brunetti *E. maxima* *E. mivati* *E. necatrix*	Fowl	Long, 1968; Patterson *et al.*, 1961

existence of intraspecific variation has come from studies made with relatively few species of parasite and is restricted almost exclusively to species of medical or veterinary importance.

1. *Trypanosome infections in cattle*

The systematic position of "breeds" of cattle is extremely confused. Although it is usually stated that there are two distinct species of cattle, *Bos tauros*, to which European breeds of cattle belong, and *Bos indicus*, the humped Zebu cattle, it is far from certain that these are in fact true species and cross breeding produces fully fertile progeny. There are a number of records of differences in the relative susceptibility of the two "species" and their hybrids to trypanosome infections and it is proposed for convenience to consider these as examples of intraspecific variation. Many breeds of domestic cattle are severely affected by trypanosome infections but it has been known for some time that certain African breeds show considerable resistance (sometimes termed "tolerance") to the pathogenic effects of infection. Among the breeds reported as "trypanosomiasis-tolerant" are N'dama, Small East African Zebu, Dwarf Shorthorn, Muturu and Nubu Mountain (Payne, 1970).

The basis of this resistance is unknown, but the possibility that it involves immunologically-mediated mechanisms has been investigated by a number of workers. Chandler (1958) compared the ability to resist trypanosome challenge of N'dama cattle that had been previously exposed to infection with that of previously uninfected animals. None of the N'dama tested showed clinical symptoms of disease, but animals previously exposed to trypanosome infection cleared parasites from the peripheral blood more rapidly and Chandler concluded that "tolerance to trypanosome infection is an inherent quality of N'dama cattle, . . . exposure to infection enhances this tolerance". More carefully controlled experiments were carried out by Roberts and Gray (1973) to compare the relative abilities of previously uninfected resistant (N'dama and Muturu) and susceptible (Zebu) breeds to withstand trypanosome challenge. Cattle of each breed were infected with trypanosomes (*T. brucei, T. congolense, T. vivax*) from wild-caught tsetse flies and the infections were terminated by chemotherapy after eight weeks. Six weeks later the cattle were challenged again.

Infections were established in all breeds from the first challenge and the levels of parasitaemia were similar; after the second challenge however N'dama cattle showed lower parasitaemias. The N'dama gained weight throughout both infections whereas Zebu lost weight, particularly during the second infection. Blood changes (erythrocyte numbers, haemoglobin levels, packed cell volumes) were less severe in N'dama than in the other breeds. None of the cattle died during these limited infections but with more intensive infections Zebu cattle did die.

Roberts and Gray (1973) were careful to distinguish between the "development of immunity" to trypanosome infection in N'dama cattle and "the ability to withstand the effects of infection" and pointed out that physiological characteristics, such as higher normal blood values (packed cell volume, haemoglobin content) and the absence of haemoglobin B, might contribute to the resistance of the breed. However, other workers (Desowitz, 1959, 1970; Weitz, 1970) have concluded that resistance does have an immunological basis and it has been shown that N'dama develop high level and long-lasting antibody responses to infection (Petit, 1974). There is evidence confirming that resistance is genetically determined, since the progeny of crosses between N'dama and Zebu cattle show a level of resistance to trypanosomiasis which is intermediate between that of the parental breeds (Chandler, 1952). The evolution of the resistant genotype in N'dama and similar breeds has obviously come about under the intense selection pressure exerted by trypanosomiasis in the African tsetse belt.

2. *Trypanosome infections in laboratory rodents*

Laboratory demonstration of intraspecific variation in response to trypanosome infection is limited and there have been no detailed analyses of the mechanisms responsible. Differences in the course of infection of *T. brucei* in two strains of outbred Swiss albino mice were recorded by Herbert and Lumsden (1967). One of the strains survived longer and showed more re-

missions of infections and it was suggested that the difference may have reflected either the relative rate of growth of the trypanosomes in the two strains or the ability of the mice to produce protective antibody.

Infection with *T. brucei* normally results in a massive increase of so-called non-specific IgM and Clarkson (1976) has made the interesting observation that there is variation between strains of mice in the extent of this increase. Of six strains studied one (C57B1) showed a marked rise in serum IgM to a level

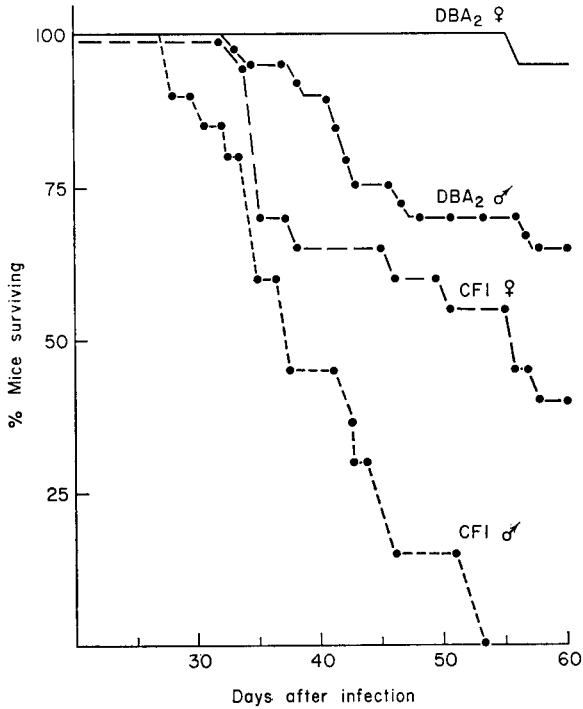

FIG. 5. Course of experimental infection with *Trypanosoma cruzi* (initiated by intraperitoneal injection) in male and female mice of two strains. (Modified from Goble, 1966.)

15 times the normal, one (C3H/mg) showed very little increase and four were intermediate. Although there was no difference in susceptibility to infection—all strains developed a heavy parasitaemia—C3H/mg mice died much earlier than did other strains. A relative resistance of C57B1 mice, in terms of survival after infection, has also been observed by Urquhart (personal communication).

The rise in serum IgM associated with trypanosome infection has been linked to the profound immunodepression that accompanies heavy parasitaemia. It is interesting therefore that Clarkson found that both C57B1 and C3H showed depression of response to injected SRBC when infected.

Differences in the susceptibility of mouse strains to *T. cruzi* has been

reported by a number of workers (Hauschka, 1947; Pizzi *et al.*, 1949; Goble, 1951; Marcuse *et al.*, 1964). In certain strains infection results in a high mortality rate, particularly in males, whereas other strains survive with relatively few deaths (Fig. 5). One reason for such variation could be the existence of interstrain differences in ability to mount an effective immune response to infection. Evidence supporting this interpretation comes from the work of Marr and Pike (1967) who found marked variation in the degree of immunity to challenge with a virulent strain of *T. cruzi*, following immunization with a strain of low virulence. Of six strains tested, four were protected by the immunizing infection and two remained susceptible. Much stronger evidence of the interrelationship between poor immunological responsiveness and susceptibility to *T. cruzi* has been obtained recently by Kierszenbaum and Howard (1976) from experimental infections in mice of the Biozzi high and low antibody-responding lines (see p. 242). Low responder mice were significantly more susceptible to infection in terms of shorter survival times, increased mortality rate, higher parasitaemias and lower LD_{50}. Mice of this line also developed much lower titres of anti-*T. cruzi* antibody ($<1 : 20$ compared with 1 : 945 in high responder mice) but, significantly, could be protected against infection by passive transfer of plasma taken from immune outbred mice. These results suggest strongly that the susceptibility of low responder line mice is a direct consequence of their deficient antibody response and does not involve any other defects in immunological reactivity. Whether this explanation holds true for other examples of strain variation in response to *T. cruzi* remains to be seen. There is evidence for the involvement of cell-mediated responses in immunity to *T. cruzi* (Mauel and Behin, 1974) and, since defects in macrophage function are known to underlie other cases of intraspecific variation in susceptibility to pathogens (see p. 246 and p. 247) the possibility should be considered that variation in macrophage activity may give rise to variation in susceptibility to *T. cruzi* as well.

3. *Malaria*

In addition to the well defined, intraspecific variation in response to malarial infection in man known to derive from innate characteristics such as haemoglobin type, there are a number of examples of variation in experimentally infected laboratory rodents that may arise from genetically determined variation in immune response. It is difficult to be certain as to the underlying mechanism because variation in host response may involve one or more of at least four possibilities: (i) variation in strain of parasite or antigenic variation occurring during the course of infection, (ii) variation in innate host characteristics, (iii) variation in non-immunological responses to infection, (iv) variation in immunological responses.

A number of workers have described variations in the course of infection of *Plasmodium berghei* within strains of mice (Kretschmar, 1962; Bruce-Chwatt, 1965), one of the most intensive studies being that of Greenberg and his coworkers (Greenberg *et al.*, 1953, 1954; Nadel *et al.*, 1955; Greenberg, 1956; Greenberg and Kendrick, 1957a,b, 1958, 1959).

These workers showed that genetically-determined variation was reflected in (i) the extent of infection in mature red blood cells and (ii) the number of mice which died at the end of the first week of infection (Table 6). It was originally hypothesized that resistance, in terms of survival, was a simple function of the inherited ability of mice to control the infection in mature red cells during the first week of infection. However, the work of Greenberg and Kendrick (1957a) suggested that the degree of parasitaemia and the extent of mortality after one week were "two partially dependent variables apparently under separate genetic control". There were thus four major categories into which strains of mice might fall; low parasitaemia—low tolerance (i.e. high mortality); low parasitaemia—high tolerance; high parasitaemia—low tolerance; high parasitaemia—high tolerance.

TABLE 6

Course of infection of Plasmodium berghei (*Kasapa strain*) *in inbred mice* (*From Greenberg and Kendrick, 1957a*)

Strain	Mean % infection of mature RBC on day 6	50% survival (days)
STR/N	5·8	12
Balb/cANN	17·1	12
C57B1/6JN	16·7	22
C3H/Hen	25·3	6
A/LN	21·1	6
DBA/2JN	36·2	6
Swiss (outbred)	49·3	6

The inheritance of resistance was studied by Greenberg and Kendrick (1958) in crosses made between Swiss (high parasitaemia) and STR (low parasitaemia) strains. It was found that the mean parasitaemia of infected F_1 progeny was intermediate between the parental values; that of the F_2 progeny was no higher than the F_1. Thus there was no evidence for single gene control of levels of parasitaemia, a conclusion in agreement with that of Nadel *et al.* (1955) and confirmed by the work of Greenberg and Kendrick (1959) on crosses made between Swiss mice and a number of inbred strains.

By relating variation in response to the level of parasitaemia in mature red cells one potential source of variation in this system, namely the availability of reticulocytes, for which *P. berghei* has a predilection, was avoided. In the earlier work, infection of mouse strains had been carried out using material taken from infected Swiss strain mice. Greenberg and Kendrick (1957b) ruled out the possibility that adaptation of *P. berghei* to particular strains had been a major factor in variation of mean parasitaemia values, although they did find that intra-strain passage within C3H mice resulted in a marked fall of parasitaemia, from 24·5%, when infection was from Swiss donors, to 3·4% when infection was from C3H donors. In contrast, intra-strain passage brought about a marked reduction in the level of mortality that occurred

during the first week of infection. Thus it is apparent that there was a very complex genetic control over the course of infection with *P. berghei* to which was added the effect of strong non-genetic factors. Even if one concentrates on the variation in initial levels of parasitaemia there are at least two sources of variation, variable immune responsiveness and differences in red cell suitability and, if one considers mortality as a criterion, then it is obvious that variability in innate tolerance to the pathogenic effects of infection must also be considered.

Variation in mouse susceptibility to *P. berghei* initiated by sporozoite as well as whole blood infection has been confirmed by Most *et al.* (1966).

Two interesting facets of intraspecific variation to *P. berghei* have been described by Friedman and Glasgow (1973) and Greenwood and Greenwood (1971). The former workers found that when random-bred CD_1 were housed individually they showed greater resistance to infection than did mice housed in groups of five, presumably as a result of the absence of stress induced by mouse–mouse interaction. No difference, however, was found in a number of other strains similarly treated. Greenwood and Greenwood (1971) followed the course of infection in New Zealand Black (NZB) and hybrid NZB $\times$ NZ White. NZB mice develop a number of spontaneous autoimmune diseases as they age, one of which is haemolytic anaemia and, as a consequence, the mice have a high proportion of reticulocytes. Infection in old (8–9 months) NZB was rapidly fatal, whereas young (1–4 months) survived. It was considered that the reticulocytosis was not a major factor in the early mortality since mice not showing autoimmune anaemia also succumbed rapidly. In all probability defective cell-mediated immunity, another consequence of aging in these mice, was responsible for the greater susceptibility of the older mice.

Selection for phenotypic resistance to *P. berghei* has been carried out by Ramakrishnan *et al.* (1964) and by Scheepers-Biva *et al.* (1967). The former workers selected from mice refractory to infection after a protocol of repeated infection and chemotherapeutic termination. Their parental generation showed 100% mortality from an untreated infection of 10^6 parasites but with each selection generation there was an increase in the prepatent period and duration of infection and a reduction in the time necessary for the induction of resistance to challenge. Scheepers-Biva *et al.* (1967) selected for seven generations and increased survival from 16% to 84%.

Zuckerman (1968) has described a similar selection for susceptibility and resistance to *P. vinckei* in rats. After 16 generations her low susceptibility line showed negligible parasitaemia from an inoculum which induced a parasitaemia of 60–70% and caused high mortality in high susceptibility rats.

4. *Leishmania*

Experimental studies of the course of infection and development of resistance to *Leishmania tropica* in the mouse have given contradictory results and variation between strains of mice has been suggested as one cause (see Mauel and Behin, 1974). The work of Bradley and Kirkley (1972) and Bradley (1974) has demonstrated such intra-strain variation in mice infected with

L. donovani. In an initial study of seven strains Bradley and Kirkley found that the response of any one strain was consistent and that it was possible to distinguish two distinct categories of response based on the growth of the parasite during the first week. In resistant strains the total liver parasite counts remained below 100 Leishman-Donovan units (LDU); in susceptible strains the count rose above 800 LDU. Bradley (1974) extended these studies to a further 18 strains and found that, without exception, strains were clearly resistant or susceptible, no overlap occurring. The inheritance of resistance and susceptibility were followed in the F_1, F_2 and backcross generations, the results suggesting strongly that resistance was controlled by a single gene or by a tightly linked group of genes (Fig. 6). No evidence of H_2-linkage or association with known *Ir* genes was found, but it was observed that resistance and susceptibility to *L. donovani* in the strains tested corresponded precisely with that to *Salmonella typhimurium.*

Bradley has concluded that this phenomenon is an example of natural resistance, since he found no evidence that resistance was related to the ability to mount an immune response, the status of resistant mice being unaltered by thymectomy. However, Plant and Glynn, who had identified genetically determined resistance and susceptibility to *S. typhimurium* in mouse strains and found a strong correlation between resistance and the ability to mount a delayed hypersensitivity response to *S. typhimurium* antigen (Plant and Glynn, 1974a), stressed that cellular immune responses were an important element in resistance to both parasites (Plant and Glynn, 1974b). Variation in immune responsiveness was, however, proposed by Bradley (see Bryceson, 1975) to explain the divergence in course of infection in susceptible strains of mice after the initial period of infection. Some strains failed to clear the infection, retained a heavy and increasing parasite burden and eventually died; others mounted a strong immune response and cleared the infection, developing strong delayed hypersensitivity. In the latter case, thymectomy did impair the protective immune response.

Intraspecific variation in response to *L. donovani* has also been described by Smrkovski *et al.* (1974). Mice of an outbred Swiss albino strain were resistant and survived infection. After ten generations of non-selective inbreeding, however, the strain response had altered and while 30% of the mice behaved similarly to the parental stock, 70% died from infection. It was suggested that there was a genetically determined defect in cell-mediated immunity in the susceptible mice since they were also shown to succumb after intravenous infection of *Mycobacterium bovis.*

5. *Coccidiosis*

It has been known for many years that breeds of chickens vary in their susceptibility to infection with species of *Eimeria*, particularly infections of *E. tenella* which can cause high mortality, and that this variability arises from inherited characteristics. As long ago as 1934, Herrick reported that selective breeding from parents which were particularly resistant to coccidial infection (*E. tenella*) produced progeny which were much more resistant than the

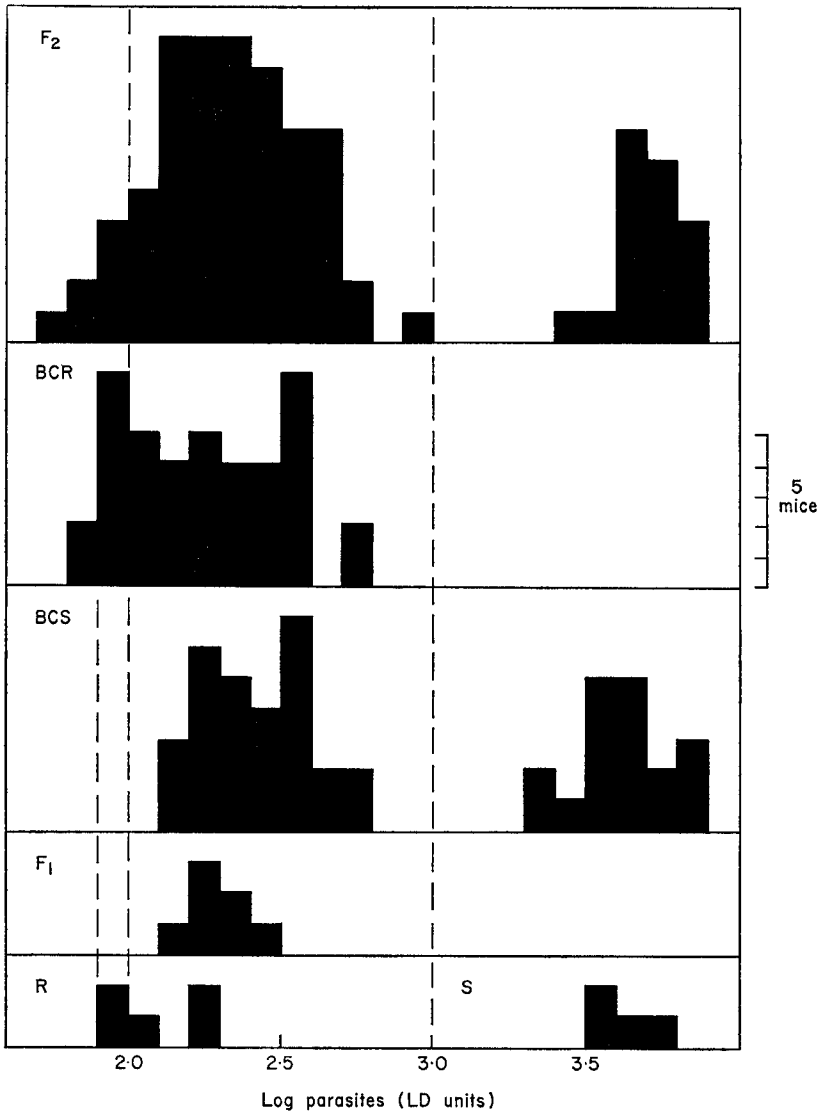

FIG. 6. Histogram showing liver parasite burdens (in LD units), 14–16 days after infection with *Leishmania donovani*, in mice derived by crossing resistant (R) and susceptible strains (S) and by backcrossing (BC) F_1 progeny to R and S parents. (Redrawn from Bradley, 1914)

progeny of unselected parents. Herrick assessed resistance by following changes in haemoglobin content and red and white blood cell counts, as well as by the numbers of chickens dying after infection. Selective breeding experiments have subsequently been performed by a number of workers, most of whom used as a criterion of resistance the ability of young chickens to survive for more than about ten days after experimental infection with standardized numbers of oocysts (see Table 7).

TABLE 7

Summary of experiments involving selective breeding for resistance and susceptibility of chickens to Eimeria tenella

% mortality after experimental infection (10^4–10^5 oocysts)			
Unselected controls	Line selected for resistance	Line selected for susceptibility	Reference
67·5	40·0	87·8	Champion, 1954
not given[a]	22·0	79·6	Jeffers, 1968
62·1	59·8[b]	84·2	Rosenberg *et al.*, 1954
62·1	14·1[c]	55·0	
34·0	15·0	72·0	Moultrie *et al.*, 1954
not given	38·0	91·0	Schildt and McGibbon, 1953

[a] Same stock as Champion. [b] Selection after 1 year. [c] Mean of subsequent 3 years.

The inheritance of resistance was analysed by Champion (1954) and Rosenberg *et al.* (1954) both of whom found that crossing resistant and susceptible parents gave F_1 progeny of intermediate resistance: Champion also found intermediate resistance in the progeny obtained by backcrossing to the resistant parent. Both workers concluded that inheritance involved multiple genetic factors which did not show complete dominance and which acted additively; sex linkage, maternal influence and cytoplasmic inheritance were considered not to be involved.

As was discussed in the introductory paragraphs of section V, the use of survival of individuals after infection as a criterion of resistance allows a variety of possible explanations. Clearly, in the examples summarized in Table 7 resistance to coccidiosis could have involved innate resistance to parasite development, innate tolerance of the mucosal destruction and haemorrhage associated with infection, or immunological responses which limited the extent of parasite proliferation. It is not possible from these reports to allocate resistance to any one of these factors although Jeffers *et al.* (1970) have stated categorically that genetic resistance to *E. tenella* "is a measure of the host's ability to withstand the lethal effects of the infection". There is some suggestion from other work that immunologically based resistance mechanisms may

play a role. Patterson *et al.* (1961) found a number of interesting corollaries of the selection for resistance and susceptibility to *E. tenella* in the University of Wisconsin short-combed White Leghorn stock which Champion (1954) had used. These workers found that the line selected for resistance to *E. tenella* was also resistant to *E. brunetti* and *E. maxima,* but showed only intermediate resistance to *E. necatrix*. The line selected for susceptibility to *E. tenella* was highly resistant to the other three species and a third selection line (line 15) was resistant to *E. tenella* but susceptible to *E. brunetti, E. maxima* and *E. necatrix*. Thus there was a marked element of specificity in resistance which might be explicable on immunological grounds.

TABLE 8

Development of immunity to Eimeria tenella *in breeds of chicken exposed to repeated infection*
(Modified from Long, 1968)

Breed	Oocyst Production ($\times 10^6$ per bird) 1st infection Total output	RI	2nd infection Total output	RI	3rd infection Total output	RI	4th infection Total output	RI
Light Sussex	27·4	54800	78·3	15670	0·07	1·4	0	0
Rhode Island Red	29·9	53900	173·4	34680	16·7	330	0	0
LS x RIR	23·2	46400	67·6	13540	0·02	0·4	0	0

$$RI = \frac{\text{no. of oocysts produced}}{\text{no. of oocysts given}}$$

Long (1968) made a detailed study on the development of *Eimeria* infections in four breeds of chicken, Rhode Island Red (RIR), Brown (BL) and White Leghorn (WL) and Light Sussex (LS). The latter proved to be more susceptible than RIR to *E. acervulina, E. brunetti, E. maxima* and *E. mivati*, but not to *E. tenella*; the Leghorn breeds were more susceptible than RIR to all species. Resistance was measured in terms of mortality, weight gain and oocyst production and Long noted large differences in oocyst output between the various breeds after a standard primary infection. There were also marked differences in the development of immunity to reinfection. RIR chickens were much slower in acquiring resistance to *E. tenella* than LS; progeny of a cross responded as did the more resistant LS parents (Table 8). Although the two breeds showed no difference in oocyst output from a primary infection of *E. tenella*, there were pronounced differences after infection with *E. maxima* and, to a lesser extent, with *E. brunetti*. Thus again there was an element of specificity in resistance. RIR chickens were more resistant than Line 151 WL to primary infection with *E. mivati* but when concurrently infected with virus responsible for Marek's disease, RIR became as susceptible as the WL (Biggs *et al.*, 1968). Concurrent infection also reduced the resistance of RIR to secondary infection with *E. mivati*. Although the authors considered that a

number of factors might contribute to this interaction, it is perhaps significant that infection with Marek's disease is known to have depressive effects on the immune response of the chicken and might therefore interfere with resistance to coccidiosis in this way.

Additional evidence for the involvement of immune responses in genetically determined resistance to coccidiosis comes from an unusual experimental approach. In recent years techniques have been developed which allow infections with *Eimeria tenella* to be established within developing chicken embryos and Long (1970) has utilized these techniques to compare the development of the parasite in embryos of three strains of White Leghorn chickens. The strains had been selected originally for susceptibility (strain "S") or resistance (strain "C" and "K") to Marek's disease, and strain "S" was found to be most susceptible also to embryonic infection with *E. tenella*, 39·7% of the embryos dying compared with 17·4% and 11·1% in "C" and "K" respectively. This pattern of susceptibility was reversed, however, when the strains were tested as 20 day chickens, strain "S" chickens being significantly more resistant than strain "C". These results may imply that the genetic differences in susceptibility measured in hatched chickens are linked to the development of immunological competence; they also raise the intriguing question of the factors responsible for the strain variation evident between infected embryos.

There appear to have been few attempts to correlate variation in resistance with other attributes of the host. Challey (1972) found that genetically resistant strains had a relatively higher corticosterone output during infection and Visco and Burns (1972) showed that susceptibility was unaltered in strains raised under bacteria-free conditions. Although the occurrence of immunity to reinfection with *Eimeria* is well established in chickens, there is still no clear picture of the mechanisms involved and it is therefore not possible to speculate on the possible relationship of specific immunological defects with the genetically determined variation that has been demonstrated.

6. *Other Protozoa*

A number of field reports have indicated that there is variation in resistance to *Babesia* infections between various breeds of cattle (Arnold, 1948; Francis, 1966; Johnston, 1967; Payne, 1970) and this has been confirmed experimentally. Daly and Hall (1955) found that following inoculation with *B. bigemina*, to which the response of cattle breeds was similar, inoculation of *B. argentina* produced only mild symptoms in pure-bred Afrikander and Zebu cattle but gave rise to severe disease in British breeds of cattle. Barnett (1963) has also described differences in response of cattle to *Theileria* infections, though he considered that variation was associated more with local selection than with breed resistance. It is obvious that variation in response to both *Babesia* and *Theileria* in the field may be markedly influenced by host variation in susceptibility to the tick vector host, an aspect which will be considered in a later section.

B. HELMINTH PARASITES

1. *Digenea*

Almost all that is known of intraspecific variation in response to digenean parasites has come from laboratory studies made with *Schistosoma mansoni* in a number of species (Table 9). *S. mansoni* will mature in a variety of hosts,

TABLE 9

Vertebrate host–parasite relationships in which genetically determined intraspecific variation in susceptibility and resistance has been demonstrated

Parasite	*Host*	*Reference*
DIGENEA		
Schistosoma mansoni	Mouse	Stirewalt, 1956; Stirewalt *et al.*, 1965
Schistosoma mansoni	Rat	Fadl, 1971
Schistosoma mansoni	Hamster	Smith and Clegg, 1976
Schistosoma mansoni	Rhesus monkey	Smithers and Terry, 1965, 1967; Cheever and Powers, 1972
Fasciola hepatica	Rat	Hughes *et al.*, 1976
CESTODA		
Echinococcus multilocularis	Mouse	Yamashita *et al.*, 1958; Lubinsky, 1964; Ali-Khan, 1974
Hymenolepis citelli	Deer mouse	Wassom *et al.*, 1974
Hymenolepis nana	Mouse	Heyneman, 1962; Thomas, 1975; Zelencov and Solonenko, 1973
Taenia taeniaeformis	Mouse	See text
Taenia taeniaeformis	Rat	Olivier, 1962a
NEMATODA		
Enterobius vermicularis	Man	Cram, 1940, 1943
Necator americanus	Man	Smillie and Augustine, 1925; Keller *et al.*, 1934, 1937; Leathers *et al.*, 1936
Ascaridia galli	Fowl	See text
Cooperia oncophora	Sheep	Kloosterman, 1974
Haemonchus contortus	Sheep	See text
Nematodirus battus	Sheep	Scrivner, 1964b
Oesophagostomum radiatum	Sheep	Seifert, 1971
Ostertagia circumcincta	Sheep	Stewart *et al.*, 1937; Scrivner, 1964a, b, 1967; Altaif, 1975
Trichostrongylus axei	Sheep	Ross, 1970

Parasite	*Host*	*Reference*
Trichostrongylus colubriformis	Sheep	Horak *et al.*, 1968
Haemonchus contortus	Goat	Warwick *et al.*, 1949
Trichostrongylus spp	Goat	Isakovich and Camacaro, 1973
Ascaris suum	Pig	Johnson *et al.*, 1975
Strongyloides ransomi	Pig	
Aspiculuris tetraptera	Mouse	Dunn and Brown, 1963
Brugia pahangi	Rat	Fox and Schacher, 1976; Sucharit and Macdonald, 1975
Litomosoides carinii	Mouse	Wenk, 1967
Nematospiroides dubius	Mouse	See text
Nippostrongylus brasiliensis	Rat	Graham and Porter, 1934; Katiyar and Sen, 1969
Nippostrongylus brasiliensis	Mouse	Ogilvie, 1971
Strongyloides ratti	Rat	Sheldon, 1937
Trichostrongylus colubriformis	Guinea pig	Rothwell, 1976
Trichuris muris	Mouse	Wakelin, 1969, 1975a, c; Worley *et al.*, 1962
Trichinella spiralis	Mouse	Duckett *et al.*, 1970; Rivera-Ortiz and Nussenzweig, 1976; Wakelin and Lloyd, 1976

but it is known that there is considerable variation in the ease with which cercariae can penetrate the skins of different animals (Stirewalt, 1963). Hence the factors which may determine susceptibility are separable into those which affect penetration (innate factors) and those which influence subsequent development (acquired factors). Stirewalt *et al.* (1965) analysed the development of infection in four strains of mice by comparing both the success of penetration of cercariae and the extent to which penetrant cercariae were able to mature. Their results showed that innate and acquired factors operated independently; hairless mice, which were least suitable for cercarial penetration, provided the most suitable environment for worm maturation. The nature of the acquired factors which were responsible for the poorer maturation of worms in other mice was not identified in this work, but it is reasonable to assume that immunological responses were involved since these are known to operate in a number of host species (Smithers and Terry, 1969).

Evidence that variation in immunological responsiveness occurs within host species has come from a number of workers, although much of it is of an indirect nature, arising incidentally from experiments designed to study other aspects of anti-schistosome immunity. Both Cheever and Powers (1965) and Smithers and Terry (1965, 1967) have commented on the unpredictable results obtained when immunizing rhesus monkeys by prior infection with cercariae

or with transferred adult worms. Some individual monkeys developed a strong immunity to challenge infection whereas in others the challenge infection developed normally. Variation has also been noted in experimental infections of rats. In a comparison of London-hooded and Wistar rats Fadl (1971) found that more worms were recovered from the former at all levels of infections given (200–1000 cercariae). In determining worm recoveries, Fadl killed the rats four weeks after infection. Since he found that London-hooded rats eliminated their worms sharply between weeks four and eight, it is possible that the difference between strains in terms of worm burden at four weeks reflected a difference in ability to generate an effective immune response, as is known to be the case in certain rodent–nematode systems (see p. 271). Smith and Clegg (1976) analysed the development of acquired immunity to *S. mansoni* in two strains of hamster and found that, whereas one strain developed a high level of resistance to challenge after a single primary infection, the other showed no statistically significant immunity to reinfection in the majority of experiments. In contrast to these reports, however, Sher *et al.* (1974) found little difference in immunity to challenge in five strains of mice.

The pathogenic consequences of schistosome infections are known to be associated with delayed hypersensitivity responses to the eggs of the parasite and thus variation in responsiveness would be expected to be reflected in variation in the severity of disease. Cheever (1965) has in fact described marked strain differences in pathology in infected mice, for example C3H mice showed considerably greater portal fibrosis and less bile duct hyperplasia than did Swiss or C57B1 mice.

Despite the very considerable volume of work on infection with *Fasciola hepatica* in a variety of hosts, it is only very recently that a report of intraspecific variation in response has appeared. Hughes *et al.* (1976) found that inbred Piebald Virol Glaxo (PVG) rats were significantly more susceptible to infection than were inbred Sprague Dawley (SD) rats in terms of the numbers of worms recoverable after three months. However, after seven to eight months the PVG rats had eliminated the flukes and were resistant to challenge, whereas SD rats still retained their primary infection. Hughes *et al.* emphasized that there may be a number of explanations for the variation between these particular strains and that the failure of SD rats to eliminate their fluke burden did not mean that the strain could be less easily immunized.

2. *Cestoda*

It was pointed out in a previous section that cestodes are, of all helminths, the group most intimately affected by the physico-chemical conditions present in their environment; variability between hosts in the course of infection may therefore reflect intraspecific variation in the innate suitability of a host for the development and maturation of the parasite. However, cestodes are known to elicit strong protective immune responses in their hosts and thus variation may also involve acquired host characteristics (Table 9). It has been accepted for some time that larval cestodes in mammalian

hosts are strongly immunogenic, but the realization that adult intestinal worms also evoke and are affected by immune responses is more recent.

A number of studies have been made of intraspecific variation in the development of larval infections of two species in particular, *Echinococcus multilocularis* and *Taeni taeniaeformis*. In both of these, infection of the intermediate host is initiated when eggs are ingested and the hexacanth penetrates the intestinal wall to complete its development within the liver; experimental infections can also be achieved by injection of cyst material. Both species can utilize a variety of rodents as their intermediate hosts, but variation has been studied almost exclusively in the mouse.

Early attempts to infect mice with *E. multilocularis* gave contradictory results and Yamashita *et al.* (1958) investigated the possibility that variation between different strains of mice might be responsible for the inconsistencies. They studied a total of ten strains and found that, while infections became established initially in all the strains, there was marked variation between strains in the proportion of mice infected, ranging from 8% to 100%. Larval development proceeded normally and give rise to infective protoscoleces in only two strains. In these strains (AKR, dba) only slight host tissue reaction was evident whereas marked reactions occurred in the eight unsuitable strains.

Lubinsky (1964) studied the growth of subcutaneously injected *E. multilocularis* cysts in seven strains of mice and found wide variation. After 120 days' growth, the weight of cysts in the most susceptible strain, C57L, was equal to 22·9% of the body weight, whereas in the least susceptible, CBA_1, cyst weight reached only 3·6% of the body weight. Not only did growth rates vary between strains, but so did the behaviour of the cysts, which became invasive in certain mice, penetrating into the abdominal and thoracic cavities. The growth of cysts in hybrid strains was also followed; in one hybrid (C57L × AHe) cyst growth was almost as great as in the most susceptible parent (C57L), in the other (C57B1 × DBA_2) growth was less in the hybrids than in either parental strain.

The susceptibility of C57 mice was subsequently confirmed by Ali Khan (1974). In the least susceptible of three strains tested (C57B1) there was a lower establishment of infection from injections of one and five cysts and a lower rate of cyst growth than in C57L. C57B1 mice also made a lower antibody response to infection, their sera giving a lower titre in an indirect haemagglutination test and fewer precipitation lines in Ouchterlony tests. Whether this differential antibody response was in any way related to the strain difference in susceptibility or merely reflected total antigenic load is impossible to say; nothing is known of the role of antibody in echinococcosis.

The development of *Echinococcus granulosus* in outbred mice has been studied by Pennoit-de Cooman and De Rycke (1970) who found some evidence suggestive of strain variation but considered that their data was not conclusive. Certainly variation where it existed was not as marked as in the mouse—*E. multilocularis* system.

Infections with the larvae (*Cysticercus fasciolaris*) of *Taenia* (= *Hydatigera*) *taeniaeformis* has been studied in strains of mice by a number of workers (Dow and Jarrett, 1960; Olivier, 1962a; Orihara, 1962; Gagarin *et al.*, 1972;

Ciccarone *et al.*, 1973) some of whom have examined strain variation in some detail. It is apparent that variation is evident less in the initial establishment and invasion of the liver than in the subsequent development and survival of the larvae. Orihara (1962) showed that, out of seven strains tested, normal development of strobilocerci took place in only two (A and AKR); in the remainder, marked tissue responses were associated with the death and disintegration of the larvae in the liver. It is interesting to observe that in contrast to the results of Yamashita *et al.* (1958—see above) with *E. multilocularis*, Orihara found dba mice to be highly resistant to *T. taeniaeformis*. Gagarin *et al.* (1972) similarly found only two of five strains to be suitable for development of strobilocerci; in the other three, larvae died after penetrating into the liver. In addition to their defined strains, Gagarin *et al.* tested wild house mice and found that individuals varied from highly susceptible to exceptionally resistant.

The most detailed study of variation between mouse strains was made by Olivier (1962a), who assessed development of larvae by size and appearance. Altogether four inbred and four outbred strains were examined and all were found to be susceptible to initial infection, although variably so. Two strains were categorized as good hosts, the larvae reaching a large size, two as reasonably good and three as uniformly poor, one outbred strain showing considerable variability (Table 10). Olivier (1962b) subsequently found that differences in susceptibility were marked only in young (< 6 weeks) mice, older mice (> 10 weeks) were all resistant in terms of larval development.

TABLE 10

Development of Taenia taeniaeformis *in different strains of mice (Modified from Olivier, 1962a)*

Category of host	Strain (*—inbred)	Results 26–31 days after infection	
		Mean no. of larvae as % of eggs fed	Mean diameter of larvae (mm)
Good	*A/LN	27·1	4·7
	C3H	32·6	3·3
Reasonably good	CFW	29·7	2·8
	Balb/c × DBA	24·1	3·6
Uniformly poor	White Swiss	9·4	1·0
	*C57 B1/6J	11·2	1·1
	*Balb/c	0·9	1·5
Variable	NIH	25·9	2·1

The basis of strain and age resistance in the mouse—*T. taeniaeformis* system was studied by treating susceptible (A/LN) and resistant (C57B1) mice with cortisone (Olivier, 1962b). Continuous administration of the drug abolished the resistance of old mice of both strains and that of young C57B1 mice, as well as further increasing the susceptibility of young A/LN mice. By

varying the time of administration of cortisone Olivier was able to show that there was a critical period between days 4 and 12 after infection at which the drug was most effective in overcoming host resistance. Limited treatment (days 0 to 16) of C57B1 mice permanently abolished resistance, larvae developing normally and surviving for up to 92 days.

These results suggest strongly that resistance to *T. taeniaeformis*, and hence intraspecific variation in this system, has an immunological basis, a view supported by the work of Ciccarone *et al.* (1973), who found that the resistance of C57B1 mice was removed by irradiation (400 rads) given 24 h before infection. In irradiated mice more than five times as many cysts developed and two-thirds of these become strobilocerci. Irradiation of C3H mice, a susceptible strain, did not alter the number of strobilocerci which developed after infection.

Olivier (1962a) also examined the susceptibility of four rat strains to *T. taeniaeformis*. As with mice, the strains showed little variation in degree of initial infection, but there were considerable differences in subsequent development. In a very detailed study, Curtis *et al.* (1933) had also shown strain differences in susceptibility of rats to infection and correlated some of these differences with the development of sarcoma in the liver.

Although it has been known for many years (Miller, 1931; Campbell, 1936) that rodents develop a strong immunity to *T. taeniaeformis* it is only recently that the underlying mechanisms have been clarified. Leid and Williams (1974) and Musoke and Williams (1975a) have shown that rats and mice respectively can be protected against infection by passive transfer of serum from infected animals; in both cases protection lay in antibodies of 7S γ fractions. Musoke and Williams (1975b) found that transferred antibody was effective after the larval cestodes had penetrated the intestinal wall, but not if given more than five days after infection, the hepatic stages rapidly becoming insusceptible to antibody. It was shown that the destruction of parasites by antibody was complement dependent and Musoke and Williams produced evidence that older larval stages released anticomplementary factors which may be responsible for their continued survival. The work of Olivier (1962b) and Ciccarone *et al.* (1973) becomes explicable in the light of this recent work, immunosuppression allowing the establishment of larvae during a critically susceptible period. One implication is, therefore, that strain variation in response to *T. taeniaeformis* may involve defects in antibody response to infection or defects in complement activity.

By comparison, there have been only a few accounts of strain variation in infection with adult tapeworms. Heyneman (1962) reported that Swiss Webster mice from two sources showed different responses to infection with *Hymenolepis nana*. In one strain there was a higher percentage recovery of cysticercoids from comparable egg infection and the adult worms were larger and more fecund. Obviously variation in this system could well have involved innate factors influencing egg hatching (see Weinmann p. 233) as well as worm growth, but it is also possible that some immunological variation may have been involved. Strain variation in the course of infections with *H. nana* has also been recorded by Zelencov and Solonenko (1973) and Thomas (1975).

One of the most significant accounts of intraspecific variation in relation to adult cestodes has come from Wassom and his colleagues (Wassom *et al.*, 1973, 1974), who have studied *Hymenolepis citelli* in both field and laboratory infections of *Peromyscus maniculatus*. Wild populations of deer mice show both a low prevalence (1·4%) and a low intensity (3·4 worms per infected host) of infection. In order to test the possibility that immune responses determined infection levels Wassom *et al.* (1973) carried out a series of laboratory infections in deer mice and found that, while the mice showed 100% susceptibility to initial infection, the majority developed a protective resistance and eliminated the adult worms. Some mice, however, failed to show this response, retaining the initial infection and being susceptible to challenge. In a subsequent paper, Wassom *et al.* (1974) carried out selective breeding experiments using both resistant and susceptible (= non-responder) animals and investigated the immunological responsiveness of each line to *H. citelli*. The major conclusions reached from this work were:

(i) Resistance is controlled by a single autosomal dominant gene.

(ii) Resistance is transferable with lymphoid cells taken from resistant donors, but not with serum.

(iii) Resistance is thymus-dependent.

The results of these experiments have important implications for both the experimental and ecological aspects of host–parasite relationships and the system would undoubtedly repay further analysis using defined inbred strains of mice in which *H. citelli* also evokes an immune response (Hopkins and Stallard, 1974).

3. *Nematoda*

Intraspecific variation in susceptibility to nematodes is, by comparison with the other groups of helminths, a relatively well documented field (Table 9). This reflects the facts that there are more host–nematode relationships available for study in the laboratory and that nematode infections are of economic significance in animals such as sheep, whose genetics have been extensively studied. It is possible also that genetically determined variability in host response may be more easily recognized when the parasite is less intimately dependent upon the host. Nematodes, unlike digeneans and cestodes, are insulated from many aspects of their environment by the cuticle and it is possible that, in these circumstances, immunologically mediated, anti-parasite activity will be more easily recognized.

(a) *Man.* Racial differences in susceptibility have been recorded on several occasions (Ackert, 1942) but for only two species does there seem to be reliable evidence that different levels of infection are determined by genetic, rather than environmental, factors. A number of reports have shown that negro races are significantly more resistant to hookworm than are white races (Smillie and Augustine, 1925; Keller *et al.*, 1934, 1937). This resistance was evident not only in the overall prevalence of infection, but also in the intensity of infection. Keller *et al.* (1937) found in North Carolina that, in populations living in the same areas, 22·1% of whites were infected, but only 4·0% of

blacks, and the intensity of infection was twice as high in the white population. Similar findings were reported by Leathers *et al.* (1936) in South Carolina. Since the hookworm in question, *Necator americanus*, originated in Africa and was taken to America by the slave trade, the differential susceptibility of the black population in America must reflect a selection for resistant genotype in the indigenous African population, although the basis of the resistance is not known.

A comparable racial difference has been demonstrated in relation to infection with the pinworm *Enterobius vermicularis* (Cram, 1940, 1943). When large samples were compared, the prevalence in white persons was 41·5% and in blacks was 12·9%. This difference was reduced when children only were compared (72% and 51%) but was similar when adults only were compared (30% and 7%). Pinworm infection is usually considered to be commonest in temperate regions and it is therefore somewhat surprising that the racial difference should be so marked.

(b) *Domestic animals.* Variation in response to nematode infection has been described in a variety of domestic animals but has been studied most extensively in chickens and sheep, particularly in the latter. Ackert and co-workers (Ackert *et al.*, 1933, 1935, 1936; Ackert and Wilmoth, 1934; Ackert, 1942) carried out a series of studies on the response of various breeds of chickens to infection with *Ascaridia galli* (= *A. lineata*) which were in fact the first experimental investigations of intraspecific variation. Using as a criterion of resistance the numbers and lengths of worms recovered after standard infections, they found pronounced differences between breeds and showed that it was possible, by selective breeding, to obtain progeny that were more resistant to infection than unselected stock. Reid (1955), however, failed to detect differences in resistance in a comparative study of native Egyptian poultry and imported standard breeds.

It has been known for many years that certain breeds of sheep, and certain individuals within breeds, survive better than others under conditions where infection with trichostrongyle nematodes is common and the phenomenon is documented in a number of field and experimental studies. In addition to reports of varying susceptibility to trichostrongyles in general (Gregory *et al.*, 1940) differential responses have been shown in relation to *Haemonchus contortus* (Whitlock, 1955, 1958; Whitlock and Madsen, 1958; Ross *et al.*, 1959; Evans *et al.*, 1963; Jilek and Bradley, 1969; Radhakrishnan *et al.*, 1972; Altaif and Dargie, 1976; Allonby and Urquhart, 1976), *Ostertagia circumcincta* (Stewart *et al.*, 1937; Scrivner, 1964a, b, 1967; Altaif, 1975), *Nematodirus battus* (Scrivner, 1964b), *Trichostrongylus colubriformis* (Horak *et al.*, 1968), *T. axei* (Ross, 1970), *Oesophagostomum radiatum* (Seifert, 1971) and *Cooperia oncophora* (Kloosterman, 1974). In the latter six species variation has been assessed primarily in terms of worm numbers, faecal egg output and host performance, but in the case of *H. contortus*, criteria of resistance have also included the ability of sheep to withstand the pathological consequences of infection, in particular to maintain a number of haematological parameters such as packed cell volume (P.C.V.) and haemoglobin content. As has been discussed earlier, such criteria may reflect either or both innate

and acquired characteristics of the host and considerable attention has been focused on the relationship between the haemoglobin type of sheep and the degree of resistance to *H. contortus*. An association between the two was first suggested by the results of Evans and Blunt (1961), who observed that the frequency of haemoglobin type A (HbA) was significantly increased in Romney Marsh sheep established in New South Wales, an area where *H. contortus* was endemic, compared to the frequency in sheep in their native British environment. Sheep of HbA type have been found to be more resistant to *H. contortus* in terms of maintaining higher haemoglobin levels and P.C.V. (Evans and Whitlock, 1964; Jilek and Bradley, 1969; Radhakrishnan *et al.*, 1972; Allonby and Urquhart, 1976; Altaif and Dargie, 1976) but the basis for the relationship has been disputed. Evans and Whitlock (1964) showed that haemoglobin type was related to erythrocyte volume and hence P.C.V., and suggested that resistance to *H. contortus* reflected a greater tolerance to the haematophagic activities of the worm. In addition, HbA is known to be associated with a number of other physiological traits (such as higher affinity for oxygen, greater capacity to increase cardiac output under anoxic stress, production of HbC under conditions of severe anaemia) which could confer greater resistance to the pathophysiological effects of infection. However, it has also been shown that HbA sheep harbour fewer worms under both field and experimental conditions (Evans *et al.*, 1963; Jilek and Bradley, 1969; Altaif and Dargie, 1976) and this has been related to a greater frequency and effectiveness of self-cure in infected sheep (Altaif, 1975; Allonby and Urquhart, 1976).

A detailed study of the response of sheep of different breeds and haemoglobin type to infection with *H. contortus* has been made recently by Altaif and Dargie (1976), who pointed out that analysis of the dynamic pathophysiological processes underlying the secondary consequences of infection was a more satisfactory way of studying the interrelationship of genetic factors and resistance. Their main conclusions were that the dominant factor in resistance to the pathological consequences of infection was variation in worm burden, which reflected an immunologically based effect upon worm establishment, and that both haemoglobin type and breed characteristics were important in this respect. Comparison of Scottish Blackface with Finn Dorset sheep showed that, within each breed, sheep of HbA were more resistant than HbB, with HbA/B intermediate, and that in general, Scottish Blackface were more resistant than Finn Dorset. Differences were most apparent when moderate larval infections were given (350 larvae kg^{-1} – see Fig. 7) but were obscured by heavier infections (1400 larvae kg^{-1}). After the smaller infection, fewer worms were recovered from Blackface than from Dorset sheep of the same Hb type and within each breed, from sheep with an HbA allele. HbA sheep showed a smaller fall in P.C.V. than did HbB, this was true also of total red cell numbers and haemoglobin concentration; HbAB sheep showed an intermediate condition. As the infection progressed the anaemia became predominantly macrocytic and hypochromic, particularly in HbB sheep. The pathophysiological studies showed that gastrointestinal haemorrhage was most severe in HbB sheep and correlated well with numbers of worms present.

Severity of haemorrhage was reflected in the degree of erythropoietic activity and the hypercatabolism of albumin in HbB sheep of both breeds and of Dorset HbB sheep in particular.

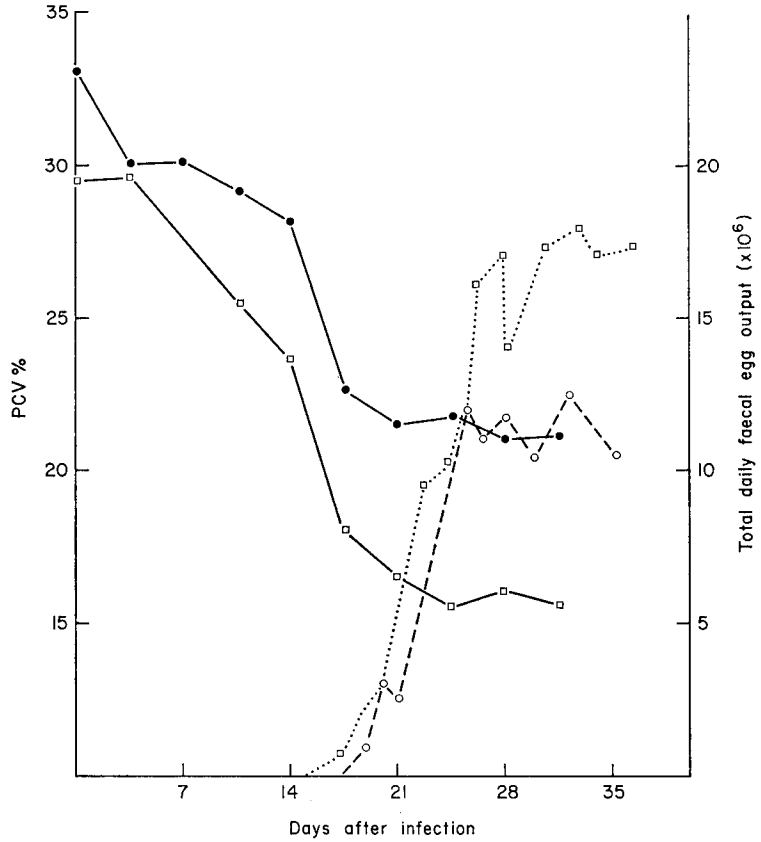

FIG. 7. Changes in packed cell volume (PCV) and total parasite egg output in Scottish Blackface sheep of different haemoglobin types infected with *Haemonchus contortus* (350 larvae kg^{-1}). (Adapted from Altaif, 1975)
Hb type A: ●——● PCV, ------ egg output
Hb type B: □———□ PCV,..... egg output

The ability of sheep to resist infection with *H. contortus* is expressed not only through mechanisms which regulate the development and maturation of worm populations, but also through the occurrence of violent hypersensitivity reactions which lead to worm expulsion (self-cure). Altaif and Dargie (1976) found that self-cure was a breed rather than a haemoglobin-type characteristic, six of eight Scottish Blackface, but only one of eight Finn Dorset showed self-cure under conditions of repeated infection. It is interesting that Altaif (1975) has shown that the form of self-cure induced by grazing on freshly growing

grass (Allonby and Urquhart, 1973) was also breed dependent, but was most effective in HbA sheep.

The study made by Altaif and Dargie is one of the few of its kind that has attempted an analysis of the factors which lead to intraspecific variation in parameters associated with infection. Their conclusion that the resistance associated with HbA stemmed ultimately from an immunologically-based capacity to control parasite burdens was made possible by the sensitivity of the techniques employed, which allowed them to monitor primary changes in host physiology associated with infection and to relate these to absolute worm numbers. It has been supported by the results of a study made of the responses of sheep of different haemoglobin types to a number of antigens, in an attempt to discover whether HbA sheep are, overall, better immune responders (Cuperlovic *et al.*, 1975). Measurement of the antibody response to rabbit red blood cells and of the antigen-binding capacity of antibody made to injected human serum albumin did in fact suggest that this was the case.

It is therefore probable that genetically determined resistance to *H. contortus* infection has a strong immunological component, which exerts an effect via control of the level of parasite establishment; non-immunological components must, however, also play an important role. Since *H. contortus* infection exerts a strong selection pressure on sheep, the distribution of HbA or HbA/B in flocks may well represent another example of a parasite-maintained polymorphism comparable to that of the sickle cell gene and malaria. Haemoglobin type may be more widely correlated with resistance to parasite infection, for example Ross (1970) showed that Dorset breed sheep (all HbA) were more resistant to *T. axei* than Scottish Blackface sheep (HbB or HbA/B) and Roberts and Gray (1973) have suggested that haemoglobin type may be relevant to the resistance of N'dama cattle to trypanosomiasis.

The existence of clearly defined differences in susceptibility of sheep to nematode infection raises the possibility of selectively breeding for progeny with increased resistance (Gregory, 1937). Whitlock (1958) and Whitlock and Madsen (1958) have shown that it is possible to breed for resistance for *H. contortus*, resistance being inherited as a simple dominant characteristic. Scrivner (1964b, 1967) successfully selected for both resistance and susceptibility to *Ostertagia circumcincta* and made the interesting observation that progeny selected for resistance to *O. circumcincta* were also resistant to infection with *H. contortus* and showed markedly lower faecal egg outputs than susceptible sheep.

Although sheep have been the most thoroughly studied domestic animal, there are a few reports of breed differences in response to nematodes in other species. Individual differences in resistance of goats to *H. contortus* infection were noted by Warwick *et al.* (1949) and the heritability of increased resistance was demonstrated in selective breeding experiments. Variation in levels of infection with *Trichostrongylus* spp. in pure bred and cross-bred goats has been recorded by Isakovich and Camacaro (1973). Johnson *et al.* (1975) compared the levels of infection in Duroc, Hampshire and Duroc × Hampshire pigs after experimental infection with *Strongyloides ransomi* and natural infection with *Ascaris suum*. All three groups showed similar sharp rises in

faecal egg output when infected with *S. ransomi*, but Duroc pigs showed the most rapid and most complete decline after four to five weeks. Hampshire pigs maintained a higher egg output than Durocs for the duration of the experiments, the cross-bred pigs showing an intermediate pattern. Breed differences in degree of infection with *A. suum* showed the reverse situation, with the cross-bred pigs again intermediate. Since the pigs used were not worm-free prior to experimental infection the precise nature of the responses to *S. ransomi* cannot be clarified, but the authors concluded that there was evidence for genetically determined differences in threshold levels and rate of response to infection. Variability in development of immunity of lambs to *Strongyloides papillosus* has been recorded by Turner (1959).

(c) *Laboratory animals.* Nematode infections in rodent hosts are extensively used as laboratory model systems and consequently intraspecific variation is known in a number of host–parasite relationships (Table 9). In the case of intestinal nematodes, host variation has most often been assessed by differences in worm burden in animals killed at particular times after infection; as such it has been recorded between individuals within random-bred strains and between members of different inbred strains. Worm burdens, however, are determined both by the level at which infections are established initially and by the effectiveness of subsequent host responses; isolated worm counts therefore throw little light on the underlying causes of variation. More detailed analysis has been carried out with relatively few species and discussion will be largely restricted to these species and to those with which selective breeding experiments have been performed.

Of the nematodes which stimulate well defined immune responses leading to expulsion of primary infections, in only two, *Trichuris muris* and *Trichinella spiralis*, has intraspecific variation in host response been studied in any detail. Surprisingly, very little is known of variation in host response to *Nippostrongylus brasiliensis*, the most extensively studied of all laboratory nematodes. Variation in rat hosts has been described specifically by Graham and Porter (1934) and by Katiyar and Sen (1969) and is also apparent in the experimental results published by a number of workers. Variation within and between strains of mice can be seen in data given by Ogilvie (1971) and it is of interest that variation was much reduced when infections were established by direct transfer of adult worms into the intestine, rather than by injection of third-stage larvae.

Although the mouse is the natural host of *Trichuris muris*, laboratory infections in random-bred strains are often characterized by marked variation in worm recovery and maturation (Keeling, 1961; Campbell, 1963); variation between strains of mice has also been recorded (Worley *et al.*, 1962). Infection of mice with *T. muris* stimulates a strong immune response, which brings about worm expulsion (Wakelin, 1967), and it is now known that the variations in worm burden that characterize infections in random-bred mice, arise as a consequence of individual differences in immune responsiveness. When mice are subjected to immunodepressive treatments this variation is minimized; by altering the times at which treatment is commenced it has been possible to show that variation in initial establishment contributes little to the

differences in worm burden that become apparent later in infection (Wakelin, 1970, 1975a).

The variation that exists among individuals of a random-bred strain is paralleled by variation between inbred strains, each of which eliminates an infection at a characteristic time. The inter-strain differences in worm burden, which become apparent when mice are killed at intervals after infection, are therefore related more to the progress of the immune response (Fig. 8) than to inherent differences in suitability for the establishment of

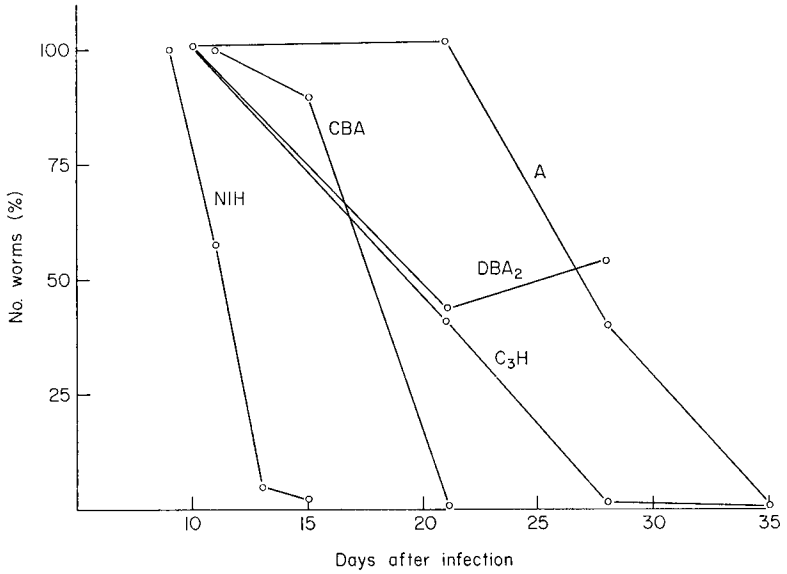

FIG. 8. Course of experimental infections with *Trichuris muris* in various inbred strains of mice.

infection. Crosses made between strains have shown that greater resistance to *T. muris* (faster worm expulsion) is inherited as a dominant characteristic, the F_1 progeny possessing the expulsion time characteristic of the parental strain with the most rapid expulsion (Wakelin, 1975a).

In this work it was observed that the strain of mice most resistant to *T. muris* responded to a lower threshold of infection than did a slower responding strain. This observation led to the suggestion that there might be genetically determined control of the level at which antigen recognition became effective and that this might operate in one or both of the components of the expulsion process, i.e. in the initial antibody-mediated phase or in the subsequent lymphoid cell-mediated phase (see Wakelin, 1975b). An alternative or additional control might be exerted on the level and timing of antibody production during the initial phase. Variation in ability to produce an antibody response could account for the wide range of strain differences in time of worm expulsion, from NIH mice which expel infections in less than 15 days to

DBA_2 mice not all of which expel worms by day 35, if it were assumed that a certain level of antibody is necessary before the cell-mediated phase of expulsion can operate. This interpretation is supported by observations that worm expulsion was accelerated in two strains of mice, characterized by rapid and slower expulsion times respectively, when immune serum was transferred (Selby and Wakelin, 1973).

Analysis of the basis of genetically determined responsiveness to *T. muris* has also been carried out in selection lines bred from the outbred Schofield strain. In unselected populations of Schofield mice a relatively constant proportion of individuals fail to expel *T. muris* and allow the worms to reach sexual maturity (Wakelin, 1969). This characteristic is easily detected and thus it has been possible to breed selectively for responsiveness and non-responsiveness (Table 11) and establish distinct lines (Wakelin, 1975c). The results

TABLE 11

Selection for resistance (responder) and susceptibility (non-responder) to *Trichuris muris* in random-bred Schofield strain mice
(Modified from Wakelin, 1975c)

Generation	Non-responder line		Responder line	
	No. of mice tested	% non-responsive	No. of mice tested	% responsive
Parental	36	28	36	72
S_1	19	30	39	95
S_2	41	27	52	100
S_3	79	75	20	100
S_4	47	79	25	100
S_5	40	88	13	100
S_6	20	75	76	100

F_1 cross 83 mice tested, 92% responsive

of the selection, together with F_1 and backcross testing, demonstrated that responsiveness was inherited as a dominant characteristic and was probably under the control of a small number of genes. As was pointed out, the use of the term "non-responder' for mice which did not expel *T. muris* did not imply total lack of response to infection. Since expulsion of *T. muris* is known to be the outcome of a series of interacting immunological events, non-responsiveness as a phenotypic character could be associated with defects in only part of the expulsion process. Infected non-responder mice do produce circulating anti-worm antibody (Wakelin, 1967) and serum taken from such mice carrying patent infections has been shown to transfer immunity to naive recipients (Wakelin, 1975d). These observations may suggest that in non-responder Schofield mice there is a defect in the cell-mediated component of expulsion, but it is equally possible that, in these mice, the production of adequate levels of protective antibody is delayed until a time at which the worms are no longer affected. Attempts to resolve this point by adoptive transfer of lymphocytes into irradiated parental mice or into responder × non-responder hybrids have

been unsuccessful and the original non-responder line has now died out because of poor breeding performance.

Intraspecific variation in response is also known to occur in mice infected with *Trichinella spiralis*. Although variations in the course of infection in this host are apparent in the data of many workers (see Wakelin and Lloyd, 1976), attribution to host, rather than to experimental, factors has been made only

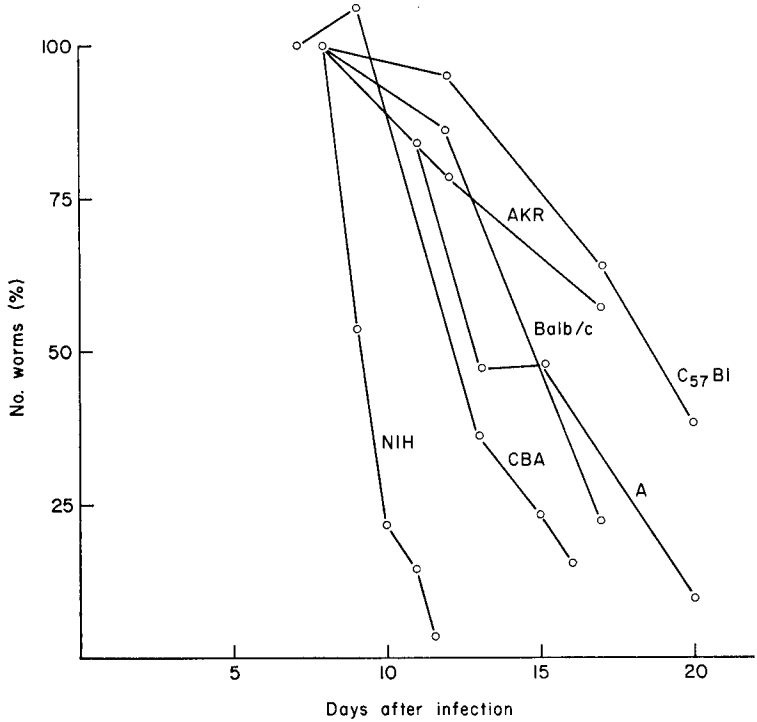

FIG. 9. Course of experimental infections with *Trichinella spiralis* in various inbred strains of mice.

recently. Duckett *et al.* (1970) described differences in the expulsion of worms from two strains of mice. Wakelin and Lloyd (1976) have analysed in some detail the response of inbred NIH strain mice, which, as in the case of *T. muris*, rapidly develop immunity and expel adult *T. spiralis* after only ten days or so, markedly earlier than almost every strain other than the normal litter mates of nude mice (Ruitenberg and Steerenberg, 1974). A study has been made (Wakelin, unpublished) of a number of mouse strains and it is apparent that a spectrum of immune responsiveness exists between inbred strains (Fig. 9) and within random-bred strains. In preliminary breeding experiments it has been found that rapidity of expulsion is inherited as a dominant characteristic, as it was also in *T. muris*.

Although the immunological and genetic mechanisms underlying strain

variation to *T. spiralis* have not yet been elucidated, an attempt has been made to determine whether immunity to this species is correlated with genetic control of antibody production. Perrudet-Badoux *et al.* (1975) followed the course of infection in two strains of mice that had been selected over many generations for differences in levels of antibody production (the Biozzi high- and low-level lines—see p. 242). Although a marked difference in anaphylactic antibody response was apparent between the two lines, no differences were found in muscle larval recoveries. It was concluded, therefore, that antibody was not involved in anti-*T. spiralis* immunity. However, the experimental design did not allow any comparison of adult worm recoveries.

More recently the relationship between anaphylactic antibody response and immunity to *T. spiralis* has been studied in a variety of mouse strains (Rivera-Ortiz and Nussenzweig, 1976). The strains showed a range of reagin response to infection and among four strains, representative of high and low responders, there were marked differences in numbers of adult worms recovered after seven days. All strains showed a similar pattern of expulsion, although obscured by intra-group variation, and the authors concluded that no correlation existed between expulsion and reagin responsiveness. However, inverse correlation was found between the level of reagin response and the number of muscle larvae recovered, SJL mice showing the lowest IgE and highest larval count, DBA_2 mice showing the converse. No conclusion was drawn about a possible causal relationship. As with previous studies of anaphylactic responses an association with H-2 type was evident (see p. 245); but it is of interest that in this case the association was made using a complex and undefined antigen (*T. spiralis* larval extract).

In contrast to *T. muris* and *T. spiralis* the nematode *Nematospiroides dubius* does not provoke a spontaneous cure response in the mouse during a primary infection, although immunity can be stimulated by prior infection and by a variety of vaccination procedures. Nevertheless, clearly defined intraspecific variation is known in this system and has received considerable attention.

A detailed study of primary infections in two inbred strains was made by Spurlock (1943). When young (32 day) animals were infected, A-W strain mice showed both a higher establishment of infection and a higher mortality than C57 mice. After infection with 500 larvae all the A-W mice were dead by day 18, whereas none of the C57 mice died within 32 days; the difference in adult worm establishment was significant but not dramatic, $478 \pm 2{\cdot}5$ and $444 \pm 2{\cdot}8$, respectively. Older (five months) mice withstood infection much better and only 2 out of 26 A-W mice died but the adult worm establishment was now higher in C57 mice ($459 \pm 2{\cdot}1$) than in A-W ($411 \pm 3{\cdot}6$). Spurlock observed that the period spent by larval stages in the intestinal mucosa was longer in A-W mice than in C57 mice and this may have contributed to the differential mortality, since the period spent in the mucosa is associated with pronounced tissue responses. It is clear, therefore, that "resistance" to *N. dubius* can be measured either by the ability to survive the effects of infection or by a reduction in the number of adult worms, the two not necessarily being coincident.

A somewhat similar picture emerges from the work of Liu (1965, 1966). In a comparative study of five strains Liu (1966) found considerable differences in resistance to the pathogenic effects of infection, infection with 600 larvae giving a LD_{50} of 14 days for the most susceptible strain (C3H) and of 40 days for the most resistant (Webster). There were no significant differences between the numbers of adult worms maturing in the two strains but the worms survived for a longer period in C3H. F_1 progeny obtained by crossing C3H and Webster mice showed greater resistance to infection (lower mortality) than either parental strain.

On histopathological evidence Liu (1965) concluded that C3H mice were "congenitally handicapped" by a lower tissue resistance to the mechanical and chemical effects of the larvae and by poorer cytopoiesis of reticuloendothelial cells in the healing process. However, some of his results suggest that the opposite was true. C3H mice showed marked polymorphonuclear infiltration only 24 hours after infection and healing of lesions was completed three days earlier than in Webster. It would appear, therefore, that greater resistance, i.e. more rapid and extensive response to larval invasion, is likely to incur a higher mortality, lower resistance favouring survival. However, it is obvious that some mortality is also associated with the adult worm populations and the reasons underlying differential mortality in mice harbouring similar numbers of worms are not readily apparent.

Immunity to challenge with *N. dubius* can be stimulated by repeated infection, but strains of mice show considerable variation in the level of protection gained and in the number of immunizing infections necessary (Cypess and Zidian, 1975). Certain strains, such as outbred ICR/CD_1 and inbred Balb/c, developed high levels of immunity after only two infections, others required at least three infections. A similar ranking of strains was apparent when mice were immunized by subcutaneous or intraperitoneal vaccination with infective larvae but one strain, C3H/HEJ, showed no immunity at all.

The results of Cypess and Zidian conflict in a number of respects with those presented by other workers. Rubin *et al.* (1971) were unable to stimulate immunity by subcutaneous injection of exsheathed larvae in two of the inbred strains used by Cypess and Zidian (C57Bl/6J and Balb/c) but achieved high levels of protection in outbred Swiss-Webster mice. Two explanations for the lack of response in the inbred mice were proposed, either that the mice were unresponsive after vaccination with excessive numbers of larvae or that the strains had tissue antigens which cross-reacted with antigens present in *N. dubius* larvae. In a later paper (Lueker and Hepler, 1975) it was shown that C57Bl/6 and Balb/c mice could be immunized by repeated oral infection, as Cypess and Zidian (1975) also found, thus it was concluded that the difference between strains lay in their capacity for immunization by the subcutaneous route.

Manger (1976) has reported success in immunizing mice of four outbred strains using an abbreviated primary infection and noted differences both in the establishment of the immunizing infection and in the degree of resistance stimulated. He failed, however, to stimulate any resistance to challenge in five inbred strains. It is apparent therefore that intraspecific variation in host

response to *N. dubius* exists but cannot always be demonstrated consistently by workers in different laboratories. Of the factors which may contribute to this situation, variation in animal management and in parasite maintenance are probably of major importance. Concurrent infections may well modify responsiveness to *N. dubius* and it is known that parasite culture conditions markedly influence virulence and immunogenicity (Hepler and Lueker, 1974).

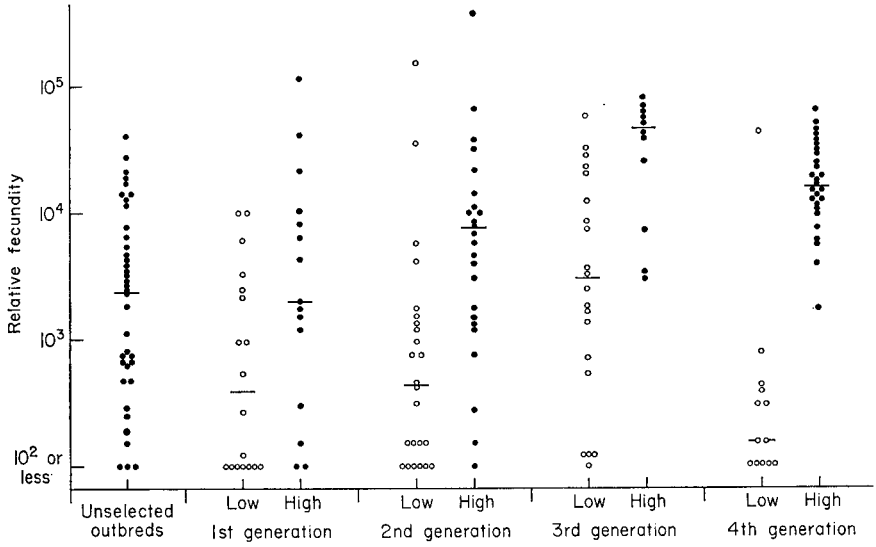

FIG. 10. Selection for susceptibility and resistance to *Trichostrongylus colubriformis* in guinea pigs. The figure shows the relative fecundity of the worm (no. eggs g^{-1} faeces × days) in individual animals of the two selection lines. (Data by kind permission of Dr. T. L. W. Rothwell)

Selective breeding of laboratory rodents for resistance and susceptibility to particular nematodes has been effected in two systems in addition to the mouse–*T. muris* system described above. Outbred guinea-pigs show considerable variation in immune response to infection with *Trichostrongylus colubriformis* and Rothwell (1976) has selectively bred for extremes of response in order to obtain distinct lines in which to study the mechanisms of immunity. To date, results are available for four generations of selection and show quite marked differences between the two lines in terms of total egg output after infection (Fig. 10). The expulsion of adult worms has also been followed in animals from the third and fourth generations. No difference in worm recovery was apparent in groups killed after seven days, but subsequently there was a sharp fall in worm count in resistant-line animals. Susceptible-line animals showed no worm loss until the third week, the mean worm counts for days 14 and 21 after infection being 1000 and 39·8, compared with 263 and 1·4 in resistant animals.

The expulsion of *T. colubriformis* from guinea-pigs is known to involve a hypersensitivity-mediated amine release and there is good evidence for a direct effect of amines upon the worms. Genetically determined differences in time of expulsion may therefore reflect an underlying control of the generation of hypersensitivity to the antigens of the worm. Rothwell has shown (1976) that the difference in susceptibility between the two selection lines was reduced when the animals were depleted of T-lymphocytes by thymectomy and anti-lymphocyte serum treatment, although both lines showed increased susceptibility.

Selection for susceptibility to *Brugia pahangi* in rats has been carried out by Sucharit and Macdonald (1973) in an attempt to develop a laboratory model for studying filarial infections. The parental stock were random-bred albino rats and selective breeding was carried out for five generations, using as far as possible pairs of animals showing positive microfilaraemia. As a result of the selection there was a considerable increase in percentage of animals in which adult worms developed after infection (16% to 71%) but a smaller increase in animals showing positive microfilaraemia (12% to 29%). Even after four generations, microfilaraemia positive individuals appeared in the progeny of microfilaraemia negative parents. It was concluded that control of susceptibility was multifactorial and was exerted in some way upon immunological mechanisms of resistance. A different approach to the problem of finding a suitable laboratory strain of host has been pursued by Fox and Schacher (1976) who have followed the course of infection of *B. pahangi* in a number of rat strains. Three strains were found to be highly susceptible to infection, two were moderately susceptible and one was resistant, the rats being negative for both microfilaraemia and adult worms. In a repeat experiment, using larger numbers of rats, the susceptibility of Lewis rats was confirmed, but the absolute resistance of Buffalo rats was not confirmed, although they appeared to be considerably less susceptible than Lewis animals.

As with so many examples of intraspecific variation, the nature of the differences between strains of rats that are susceptible or resistant to filariids is not known. Although Sucharit and Macdonald suggested that immunological factors were important, it is of course possible that innate factors contribute as well. Wenk (1967) has shown, for example, that in different strains of mice infected with *Litomosoides carinii* there were quite considerable differences in the number of larvae that migrated successfully from the infection site to the pleural cavity. Since very little is understood of immunity to filarial infection, the mechanisms which may be under genetic control can only be guessed at.

C. ARTHROPOD PARASITES

Ectoparasitic blood-feeding arthropods are known to stimulate a variety of changes in the skin of the host (Benjamini and Feingold, 1970) which in many cases render the host unsuitable for further feeding, i.e. the host acquires resistance. Immunologically mediated, immediate and delayed hypersensitivity responses, initiated by antigens present in salivary and other secretions

of the parasite, are major components of such changes (Trager, 1939; Riek, 1962; Allen, 1973; Wikel and Allen, 1976) and thus there is strong presumptive evidence that the numerous recorded instances of intraspecific variation in response to arthropod infection involved genetically determined variation in immune responsiveness (Table 12).

TABLE 12

Vertebrate host–parasite relationships in which genetically determined intraspecific variation in susceptibility and resistance has been demonstrated

Parasite	*Host*	*Reference*
ARTHROPODA		
Arachnida		
Ixodid ticks	Cattle	Francis and Little, 1964
Boophilus microplus	Cattle	Wilkinson, 1955; Riek, 1962; Roberts, 1968
Ornithonyssus sylviarum	Fowl	Hall and Gross, 1975
Insecta		
Melophagus ovinus	Sheep	Nelson, 1962; Nelson and Bainborough, 1963
Polyplax serrata	Mouse	Bell *et al.*, 1966; Clifford *et al.*, 1967

Breeds of cattle are known to vary in susceptibility to tick infestation; for example, British breeds such as Herefords, Friesians and Shorthorns become more heavily infected than Zebu type cattle or Zebu–British cross-breds (Riek, 1962; Francis and Little, 1964; Roberts, 1968). Although it has been suggested that innate factors such as skin and coat characteristics may contribute to such variation (Francis and Little, 1964) other workers have been unable to find a correlation (Wilkinson, 1955; Riek, 1962). As part of a detailed series of studies on the reactions of animals to tick infestation, Riek (1962) examined the resistance of Shorthorn, Zebu and Shorthorn × Zebu cross-bred cattle to the tick *Boophilus microplus*. He found some evidence of an innate resistance in Zebu cattle but concluded that acquired resistance, manifested in hypersensitivity reactions to antigens from tick saliva, was the most important element in tick resistance. Shorthorn cattle showed poorer acquired responses than the cross-breds but there was considerable individual variation within each breed. Individual differences in resistance were also noted by Wilkinson (1955) and, in a later study of resistance of cattle to *Boophilus microplus*, Roberts (1968) concluded that each animal was capable of developing a particular level of resistance, thus harbouring a certain number of ticks, and that the acquisition of immunity merely meant that the level was reached more quickly. Roberts quoted evidence that an individual's level of resistance is an inherited characteristic (Wharton and Utech, personal communication).

Intraspecific variation in response to parasitic insects has also been described. Nelson (1962) and Nelson and Banborough (1963) observed that

individual sheep vary in their ability to manifest resistance to the ked, *Melophagus ovinus*, again concluding that resistance was mediated by parasite-induced changes in the host's skin. A detailed study of resistance and variation in strains of mice infected with the louse, *Polyplax serrata*, was undertaken by Bell *et al.* (1966) and Clifford *et al.* (1967). Mice are normally able to control the level of infection by grooming but, when disabled by removal of limbs, may become heavily infested. Characteristically the infection shows a pattern of population increase and subsequent decrease. In

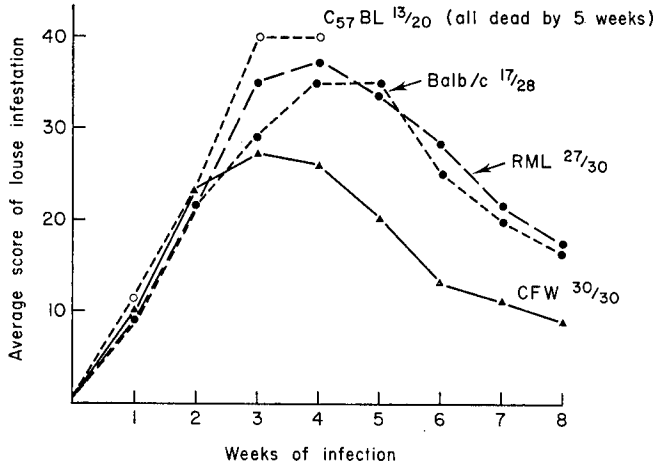

FIG. 11. Susceptibility of various strains of mice to infection with the louse *Polyplax serrata*. Numbers after each strain indicate number of mice surviving out of those infected. Score of louse infestation = an arbitrary scale in which 10 = rare, 20 = few to moderate, 30 = many, 40 = very many. (Modified from Clifford *et al.*, 1967)

earlier work it had been noted that when groups of disabled albino RML mice were infected there were, in every group, individual mice which developed heavy and protracted infections and others that supported only low and transitory populations. Clifford *et al.* (1967) studied infections in a number of mouse strains and found that there were considerable differences in susceptibility, the differences being apparent in the level of infestation reached and in the number of mice dying (Fig. 11). C57B1 mice were found to be most susceptible and showed a high mortality. By removing the infestation with insecticide treatment Clifford *et al.* were able to show that susceptible mice had developed no resistance to reinfection.

The mode of inheritance of resistance was studied by crossing susceptible and resistant strains and the results indicated that resistance was inherited as a dominant characteristic (Clifford *et al.*, 1967). A short-term selection experiment, using resistant individuals of the RML strain, failed to show an increased resistance in the progeny, but only two generations were studied. Overall the breeding experiments suggest a polygenic control.

The nature of resistance to *Polyplax* was not determined, but it was shown by Bell *et al.* (1966) that resistance appeared to operate locally at the site of infestation rather than generally over the whole body. It was considered that environmental changes, for example, accumulation of detritus, were not major factors in resistance; an immunologically mediated resistance would therefore seem to be involved. It is interesting to note that genetically determined variation in response to *Polyplax* was only evident in the absence of grooming, an essentially non-specific defence mechanism.

If hypersensitivity reactions are the underlying factor in resistance and thus in variation, then there are a number of levels at which genetic control could operate, from T-cell function to phagocytic and amine-releasing cell activity. A non-specific defect in regulation of antibody-mediated hypersensitivity could conceivably be involved, certainly in man it is commonly observed that individuals vary markedly in allergic response to ectoparasite bites (see Benjamini and Feingold, 1970).

VII. Conclusions—The Implications of Intraspecific Variation in Response to Infection

The main purpose of this review was to document as fully as possible the occurrence of genetically determined, intraspecific variation in host response to parasitic infection and to point to some of the factors through which genetic control might operate. However, the existence and the ubiquity of the phenomenon raise issues relevant to many aspects of parasitology and the review would be incomplete if some of these were not to be considered, however briefly.

Ecology of Parasites

Comparatively little is known of the factors which regulate the population dynamics of natural infections and the role of immune responses in determining overall levels of transmission and infection has not received a great deal of attention (Bradley, 1972; Kennedy, 1975; Wakelin, 1976). However, there is limited evidence that wild strains of hosts can develop levels of immunity comparable to those shown in laboratory models of infection (Behnke and Wakelin, 1973; Wassom *et al.*, 1974) and thus individuals which for any reason are incapable of responding must play an important part in the transmission and survival of the parasite concerned. Variation in response may have a physiological basis and thus be evident within groups of the host population defined by age, sex or reproductive status, or have a genetic basis and thus occur throughout the population. Since natural populations are composed of outbred individuals it is reasonable to assume that they will show the degree of variation in immune responsiveness that laboratory populations show and this supposition is supported by experimental infections of wild mice with *Taenia taeniaeformis* (Gagarin *et al.*, 1972) and *Trichuris muris* (Behnke and Wakelin, 1973) and of deer mice with *Hymenolepis citelli*

(Wassom *et al.*, 1974). The existence of genetically determined low responsiveness or unresponsiveness to infection could be an important factor in bringing about the overdispersion so characteristic of parasite populations, when the majority of parasites are found in a small number of the infected hosts. It may also help to explain the focal nature of many parasitic infections, such as those in small rodents, since transmission would be most effective only within the range of the infected individuals. This possibility was discussed by Wassom *et al.* (1974) in the context of proven host variability in the response of deer mice to *Hymenolepis citelli* and has been raised as one of a number of explanations for the focal distribution of *Capillaria hepatica* (Layne and Griffo, 1961).

Evolutionary Aspects

The existence of intraspecific differences in resistance and susceptibility will have evolutionary consequences for both members of a host–parasite relationship. The importance of parasites in maintaining polymorphism in the host population has already been mentioned in this review and has been discussed at some length by Damian (1964); where selection by the parasite is intense, resistant genotypes may spread throughout the whole population (Rodriguez and Craig, 1973; Bartlett and Ball, 1966). Equally, the parasite is capable of responding to strong selection pressure and survival in less resistant members of an otherwise resistant population may allow the evolution of strains which are not rejected by the host. One of the consequences of this interaction is the evolution of strain–strain specificity in certain host–parasite relationships, for example schistosomes in their intermediate hosts, but another could be the extension of the range of hosts parasitized.

Experimental Studies of Immunity

Intraspecific variation in response to infection emphasizes the importance of using precisely defined strains of host species and of selecting animals whose immunological characteristics are suitable for the experimental design in question. The use of inbred animals, where possible, reduces some of the variability inherent in experimental host–parasite systems and removes one of the difficulties in demonstrating differential responses, particularly where these are qualitatively or quantitatively small. When it is not possible to use inbred strains, the existence of clearly defined differences between outbred individuals in response to infection should be allowed for. Equally, recognition of interstrain differences in resistance or susceptibility should caution against extrapolation of results obtained with one particular strain. The parameters which define responses to infection are frequently characteristic of a host strain rather than of the host species and must be ascertained for each strain used.

Knowledge of the inherited nature of intraspecific variation raises the possibility of selectively breeding lines of animals with clearly differentiated responses to a particular infection, so that the underlying mechanisms of

resistance can be analysed. As this review has shown, such an approach has been used with a variety of parasites in both invertebrate and vertebrate hosts. Additionally, there is now the possibility of using as experimental hosts, animals with a wide range of genetically determined, immunological peculiarities. The use of the athymic nude mouse as a host lacking T-cell responsiveness is an extreme example but one can point also to the recent use of the Biozzi lines of high- and low-level antibody poducing mice in the study of immune responses to *Trypanosoma cruzi* (Kierszenbaum and Howard, 1976) and *Trichinella spiralis* (Perrudet-Badoux *et al.*, 1975).

Control of Parasitic Diseases

In a symposium devoted to consideration of the future of nematology Stoll (1962) remarked "It does not seem likely we will long continue almost to neglect the study of the genetics of the host in relation to its parasitic nematodes. In animal husbandry, for instance, the breeding of strains more resistant to nemas could conceivably be more rewarding than preoccupation with anthelmintics". Stoll's optimism remains largely unfulfilled and there are a number of reasons for caution about the practicability of breeding resistant strains. Perhaps the major obstacle is the necessity for animals to be economically profitable and thus for parasite resistance to be linked to other characteristics such as wool growth, milk yield and meat production. Nevertheless, the substantial evidence that genotypes exist which confer resistance to such economically important parasitic diseases as coccidiosis, trypanosomiasis and haemonchosis merits serious investigation. Surprisingly little is known of the basis of resistance in such cases and analysis of the immunological factors involved may be useful both in understanding the pathogenesis of disease and in determining whether it is possible, by hybridization or by selection, to confer resistance upon strains which possess other desirable characteristics.

As far as medical parasitology is concerned, a knowledge and understanding of the basis of racial and individual variation in susceptibility should contribute to the development of effective control measures, whether chemotherapeutic or immunoprophylactic. An important and, as yet, relatively unstudied field is the association of an individual's genetically determined immune responsiveness to parasitic infection with the generation of pathological changes. Here too, an understanding of the association might lead to measures designed to lessen the severity of such changes.

As this review has shown, there have been many descriptive accounts of intraspecific variation in susceptibility and resistance to parasitic infection, but relatively few analyses of the mechanisms of control or the means of expression of such variation. If, by drawing attention to this aspect of the host–parasite relationship, the review stimulates interest and further research, then it will have served a useful purpose, as there are undoubtedly valuable contributions that an understanding of intraspecific variation can make to the subject of parasitology as a whole.

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References

Ackert, J. E. (1942). Natural resistance to helminthic infections. *Journal of Parasitology* **28**, 1–24.

Ackert, J. E. and Wilmoth, J. H. (1934). Resistant and susceptible strains of white Minorca chickens to the nematode, *Ascaridia lineata* (Schneider). *Journal of Parasitology* **20**, 323–324.

Ackert, J. E., Eisenbrandt, L. L., Glading, B. and Wilmoth, J. H. (1933). On the comparative resistance of six breeds of chickens to the nematode *Ascaridia lineata* (Schneider). *Journal of Parasitology* **20**, 127.

Ackert, J. E., Eisenbrandt, L. L., Wilmoth, J. H., Glading, B. and Pratt, I. (1935). Comparative resistance of five breeds of chickens to the nematode *Ascaridia lineata* (Schneider). *Journal of Agricultural Research* **50**, 607–624.

Ackert, J. E., Pratt, I. and Freeman, A. E. (1936). Resistant and susceptible groups of white leghorn chickens to the nematode *Ascaridia lineata* (Schneider). *Anatomical Records* **67**, (1 Suppl) 130.

Ali-Khan, Z. (1974). Host–parasite relationship in echinococcosis. I. Parasite biomass and antibody response in three strains of inbred mice against graded doses of *Echinococcus multilocularis* cyst. *Journal of Parasitology* **60**, 231–235.

Allen, J. R. (1973). Tick resistance: basophils in skin reactions of resistant guinea pigs. *International Journal of Parasitology* **3**, 195–200.

Allison, A. C. (1954). Protection afforded by sickle-cell trait against subtertian malaria. *British Medical Journal* **1**, 290–294.

Allison, A. C. (1957). Malaria in carriers of the sickle-cell trait and in newborn children. *Experimental Parasitology* **6**, 418–447.

Allison, A. C. (1963). Inherited factors conferring resistance to protozoa. *In* "Immunity to Protozoa" (P. C. C. Garnham, A. E. Pierce and I. Roitt, eds), pp, 109–122. Blackwell Scientific Publications, Oxford.

Allison, A. C. (1964). Polymorphism and natural selection in human populations. *Cold Spring Harbor Symposium of Quantitative Biology* **29**, 137–149.

Allison, A. C. (1965). Genetic factors in resistance against virus infections. *Archiv für die gesamte Virusforschung* **17**, 280–294.

Allison, A. C. (1975). Interactions of genetic predisposition, acquired immunity and environmental factors in susceptibility to disease. *In* "Man-made Lakes and

Human Health" (N. F. Stanley and M. P. Alpers, eds), pp. 401–420. Academic Press, London and New York.

Allonby, E. W. and Urquhart, G. M. (1973). Self-cure of *Haemonchus contortus* under field conditions. *Parasitology* **66**, 43–53.

Allonby, E. W. and Urquhart, G. M. (1976). A possible relationship between haemonchosis and haemoglobin polymorphism in Merino sheep in Kenya. *Research in Veterinary Science* **20**, 212–214.

Alper, C. A. and Rosen, F. S. (1971). Genetic aspects of the complement system. *Advances in Immunology* **14**, 251–290.

Alper, C. A. and Rosen, F. S. (1974). The role of complement *in vivo* as revealed by genetic defects. *In* "Progress in Immunology II" (L. Brent and J. Holborow, eds), Vol. I, pp. 201–208. North-Holland Publishing Co., Amsterdam.

Alpers, J. H., Steward, M. W. and Soothill, J. F. (1972). Differences in immune elimination in inbred mice. The role of low affinity antibody. *Clinical and Experimental Immunology* **12**, 121–132.

Altaif, K. I. (1975). "Genetic resistance of helminth infections in sheep". Ph.D. Thesis, University of Glasgow.

Altaif, K. I. and Dargie, J. D. (1976). Genetic resistance of sheep to *Haemonchus contortus*. "Proc. Int. Symp. in Nuclear Techniques in Animal Production and Health." pp. 449–462. I.A.E.A., Vienna.

Amsbaugh, D. F., Hansen, C. T., Prescott, B., Stashak, P. W., Barthold, D. R. and Baker, P. J. (1972). Genetic control of the antibody response to type III Pneumococcal polysaccharide in mice. I. Evidence than an X linked gene plays a decisive role in determining responsiveness. *Journal of Experimental Medicine* **136**, 931–949.

Armerding, D., Katz, D. H. and Benacerraf, B. (1974). Immune response genes in inbred rats. II. Segregation studies of the GT and GA genes and their linkage to the major histocompatibility locus. *Immunogenetics* **1**, 340–351.

Arnold, R. M. (1948). Resistance to tick-borne disease. *Veterinary Record* **60**, 426.

Bang, F. B. and Warwick, A. (1960). Mouse macrophages as host cells for the mouse hepatitis virus and the genetic basis of their susceptibility. *Proceedings of The National Academy of Sciences* USA **46**, 1065–1075.

Barbosa, F. S. and Barreto, A. C. (1960). Differences in susceptibility of Brazilian strains of *Australorbis glabratus* to *Schistosoma mansoni*. *Experimental Parasitology* **9**, 137–140.

Barnett, S. F. (1963). The biological races of the bovine *Theileria* and their host-parasite relationship. In "Immunity to Protozoa" P. C. C. Garnham, A. E. Pierce and I. Roitt, eds), pp. 180–195. Blackwell Scientific Publications, Oxford.

Barr, R. A. (1975). Evidence for genetical control of invertebrate immunity and its field significance. In "Invertebrate Immunity" K. Maramorosch and R. E. Shope eds), pp. 129–135. Academic Press, London and New York.

Bartlett, B. R. and Ball, J. C. (1966). The evolution of host suitability in a polyphagous parasite with special reference to the role of parasite egg encapsulation. *Annals of the Entomological Society of America* **59**, 42–45.

Basch, P. F. (1975). An interpretation of snail–trematode infection rates: specificity based on concordance of compatible phenotypes. *International Journal of Parasitology* **5**, 449–452.

Basch, P. F. (1976). Intermediate host specificity in *Schistosoma mansoni*. *Experimental Parasitology* **39**, 150–169.

Beckett, E. B. and MacDonald, W. W. (1971). The survival and development of

subperiodic *Brugia malayi* and *B. pahangi* larvae in a selected strain of *Aedes aegypti*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **65**, 339–346.

Behnke, J. M. and Wakelin, D. (1973). The survival of *Trichuris muris* in wild populations of its natural hosts. *Parasitology* **67**, 157–164.

Bell, J. F., Clifford, C. M., Moore, G. J. and Raymond, G. (1966). Effects of limb disability on lousiness in mice. III Gross aspects of acquired resistance. *Experimental Parasitology* **18**, 49–60.

Benacerraf, B. and Dorf, M. (1974). Genetic control of specific immune responses. *In* "Progress in Immunology II" (L. Brent and J. Holborrow, eds), Vol. 2, pp. 181–191. North-Holland Publishing Co., Amsterdam.

Benacerraf, B. and Katz, D. H. (1975). The histocompatibility-linked immune response genes. *Advances in Cancer Research* **21**, 121–173.

Benacerraf, B. and McDevitt, H. O. (1972). Histocompatibility-linked immune response genes. *Science, New York* **175**, 273–279.

Benjamini, E. and Feingold, B. F. (1970). Immunity to arthropods. *In* "Immunity to Parasitic Animals" (G. J. Jackson, R. Herman and I. Singer, eds), Vol. 2, pp. 1061–1134. Appleton-Century-Crofts, New York.

Biggs, P. M., Long, P. L., Kenzy, S. G. and Rootes, D. G. (1968). Relationship between Marek's disease and coccidiosis. II. The effect of Marek's disease on the susceptibility of chickens to coccidial infection. *Veterinary Record* **83**, 284–289.

Biozzi, G., Asofsky, R., Lieberman, R., Stiffel, C., Mouton, D. and Benacerraf, B. (1970). Serum concentrations and allotypes of immunoglobulins in two lines of mice genetically selected for "high" or "low" antibody synthesis. *Journal of Experimental Medicine* **132**, 752–764.

Biozzi, G., Stiffel, C., Mouton, D., Bouthillier, Y. and Decreusefond, C. (1971). Genetic regulation of the function of antibody producing cells. *In* "Progress in Immunology" (B. Amos, ed.), pp. 529–545. Academic Press, London and New York.

Blaese, R. M., Strober, W., Brown, R. S. and Waldmann, T. A. (1968). The Wiskott-Aldrich syndrome, a disorder with a possible defect in antigen processing or recognition. *Lancet* **i**, 1056–1060.

Blanden, R. V., Hapel, A. J. and Jackson, D. C. (1976). Mode of action of Ir genes and the nature of T cell receptors for antigen. *Immunochemistry* **13**, 179–191.

Blomberg, B., Geckeler, W. R. and Weigert, M. (1972). Genetics of the antibody response to dextran in mice. *Science, New York* **177**, 178–180.

Bradley, D. J. (1972). Regulation of parasite populations. A general theory of the epidemiology and control of parasitic infections. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **66**, 697–708.

Bradley, D. J. (1974). Genetic control of natural resistance to *Leishmania donovani* in mice. *Nature, London* **250**, 353.

Bradley, D. J. and Kirkley, J. (1972). Variation in susceptibility of mouse strains to *Leishmania donovani* infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **66**, 527–528.

Bray, R. S. (1958). The susceptibility of Liberians to the Madagascar strain of *Plasmodium vivax*. *Journal of Parasitology* **44**, 371–373.

Brewer, G. J. and Powell, R. D. (1965). A study of the relationship between the content of adenosine triphosphate in human red cells and the course of falciparum malaria: a new system that may confer protection against malaria. *Proceedings of the National Academy of Sciences USA* **54**, 741–745.

Brown, K. N. (1976). Specificity in host–parasite interaction. *In* "Receptors and Recognition" (P. Cuatrecasas and M. F. Greaves, eds), Vol. I, pp. 118–175. Chapman and Hall, London.

Bruce-Chwatt, L. J. (1965). Les souches de souris resistantes au *Plasmodium berghei*. *Annales de la Société Belge de Médecine tropicale* **45**, 299–312.

Bryceson, A. D. M. (1975). Mechanisms of disease in leishmaniasis. Thirteenth Symposium of the British Society for Parasitology ("Pathogenic Processes in Parasitic Infection"). pp. 85–100. Blackwell Scientific Publications, Oxford.

Buckley, C. E., Dorsey, F. C., Corley, R. D., Ralph, W. B., Woodbury, M. A. and Amos, D. B. (1973). HL-A-linked human immune response genes. *Proceedings of the National Academy of Sciences USA* **70**, 2157–2161.

Campbell, D. H. (1936). Active immunization of albino rats with protein fractions from *Taenia taeniaeformis* and its larval form *Cysticercus fasciolaris*. *American Journal of Hygiene* **23**, 104–113.

Campbell, W. C. (1963). Spontaneous cure in *Trichuris muris* infections in albino mice and its suppression by cortisone. *Journal of Parasitology* **49**, 628–632.

Cuperlovic, K., Altaif, K. I., Dargie, J. D. and Mulligan, W. (1975). A possible relationship between haemoglobin polymorphism and the immune response of sheep. *Proceedings of the Second European Multicolloquium Parasitology* (*Trogir*).

Cepellini, R. (1972). Associations between HL-A type and specific disease entities. *In* "Genetic Control of Immune Responsiveness" (H. O. McDevitt and M. Landy, eds), pp. 356–357. Academic Press, London and New York.

Challey, J. R. (1966). Changes in adrenal constituents and their relationship to corticosterone secretions in chickens selected for genetic resistance and susceptibility to caecal coccidiosis. *Journal of Parasitology* **52**, 967–974.

Champion, L. R. (1954). The inheritance of resistance to cecal coccidiosis in the domestic fowl. *Poultry Science* **33**, 670–681.

Chandler, R. L. (1952). Comparative tolerance of West African N'dama cattle to trypanosomiasis. *Annals of Tropical Medicine and Parasitology* **46**, 127–134.

Chandler, R. L. (1958). Studies on the tolerance of N'dama cattle to trypanosomiasis. *Journal of Comparative Pathology* **68**, 253–260.

Cheever, A. W. (1965). A comparative study of *Schistosoma mansoni* infections in mice, gerbils, multimammate rats and hamsters. *American Journal of Tropical Medicine and Hygiene* **14**, 227–238.

Cheever, A. W. and Powers, K. G. (1972). *Schistosoma mansoni* infection in rhesus monkeys: comparison of the course of heavy and light infection. *Bulletin of the World Health Organization* **46**, 301–309.

Ciccarone, P., Ferretti, G., Gabriele, F. and Marconi, C. (1973). [Development of larval forms of *Hydatigera taeniaeformis* in pre-irradiated mice.] *Parassitologia* **15**, 314–315. Abstracted in *Helminthological Abstracts* 1975, **55**, 1886.

Clark, E. W. and Ball, G. H. (1952). The free amino acids in the whole bodies of culicid mosquitoes. *Experimental Parasitology* **1**, 339–346.

Clarke, B. (1976). The ecological genetics of host–parasite relationships. Fourteenth Symposium of the British Society for Parasitology ("Genetic Aspects of Host–Parasite Relationships"). pp. 87–103. Blackwell Scientific Publications, Oxford.

Clarkson, M. J. (1976). IgM in *Trypanosoma brucei* infection of different strains of mice. *Parasitology*, **73**, viii.

Clifford, C. M., Bell, J. F., Moore, G. J. and Raymond, G. (1967). Effects of limb disability on lousiness in mice. IV Evidence of genetic factors in susceptibility to *Polyplax serrata*. *Experimental Parasitology* **20**, 56–67.

Cohen, S. and Sadun, E. H. (eds) (1976). "Immunology of Parasitic Infections." Blackwell Scientific Publications, Oxford.

Coutts, W. and Silva-Inzunza, E. (1957). Quoted by Honigberg, 1970.

Cram, E. B. (1940). Studies on oxyuriasis. XXIV Comparative findings in the white and negro races. *Proceedings of the Helminthological Society of Washington* **7**, 31–35.

Cram, E. B. (1943). Studies on oxyuriasis. XXVIII Summary and conclusions. *American Journal of Disease of Children* **65**, 46–59.

Cuckler, A. C. (1970). Coccidiosis and histomoniasis. *In* "Immunity to Parasitic Animals" (G. J. Jackson, R. Herman and I. Singer, eds), Vol. 2, pp. 371–397. Appleton-Century-Crofts, New York.

Curtis, M. R., Dunning, W. F. and Bullock, F. D. (1933). Genetic factors in relation to the etiology of malignant tumours. *American Journal of Cancer* **17**, 894–923.

Cypess, R. H. and Zidian, J. L. (1975). *Heligmosomoides polygyrus* (= *Nematospiroides dubius*): the development of self-cure and/or protection in several strains of mice. *Journal of Parasitology* **61**, 819–824.

Daly, G. D. and Hall, W. T. K. (1955). A note on the susceptibility of British and some Zebu type cattle to tick fever (Babesiosis). *Australian Veterinary Journal* **31**, 152.

Damian, R. T. (1962). A theory of immunoselection for eclipsed antigens of parasites and its implications for the problem of antigenic polymorphism in Man. *Journal of Parasitology* **48**, (Sect. 2), 16.

Damian, R. T. (1964). Molecular mimicry: antigen sharing by parasite and host and its consequences. *American Naturalist* **98**, 129–149.

Darnell, M. G., Koprowski, H. and Lagerspetz, K. (1974). Genetically determined resistance to infection with Group B Arboviruses. I. Distribution of the resistance gene among various mouse populations and characteristics of gene expression. *Journal of Infectious Diseases* **129**, 240–247.

Davidson, I. and Stern, K. (1954). Heterohaemoantibodies in inbred strains of mice. *Journal of Immunology* **72**, 216–223.

Debre, P., Kapp, J. A. and Benacerraf, B. (1975a). Genetic control of specific immune suppression. I. Experimental conditions for the stimulation of suppressor cells by the co-polymer L-glutamic acid ^{50}-L-tyrosine^{50} (GT) in non-responder Balb/c mice. *Journal of Experimental Medicine* **142**, 1436–1446.

Debre, P., Kapp, J. A., Dorf, M. E. and Benacerraf, B. (1975b). Genetic control of specific immune suppression. II. H-2 linked dominant genetic control of immune suppression by the random copolymer L-glutamic acid ^{50}-L-tyrosine^{50} (GT). *Journal of Experimental Medicine* **142**, 1447–1454.

de Souza, C. M., Sauerbronn Maia, L. C. and Vaz, N. M. (1974). Susceptibility to cutaneous anaphylaxis in inbred strains of mice. *Journal of Immunology* **112**, 1369–1372.

Desowitz, R. S. (1959). Studies on immunity and host–parasite relationships. I. The immunological response of resistant and susceptible breeds of cattle to trypanosomal challenge. *Annals of Tropical Medicine and Parasitology* **53**, 293–313.

Desowitz, R. S. (1970). African trypanosomes. *In* "Immunity to Parasitic Animals" (G. J. Jackson, R. Herman and I. Singer, eds), Vol. 2, pp. 551–596. Appleton-Century-Crofts, New York.

Desowitz, R. S. and Challappah, W. J. (1965). Fate of transplanted third stage *Brugia pahangi* larvae in normally susceptible and non susceptible mosquito hosts. *Journal of Parasitology* **51**, 195–199.

Dewitt, W. B. (1954). Susceptibility of snail vectors to geographic strains of *Schistosoma japonicum*. *Journal of Parasitology* **40**, 453–456.

Dineen, J. K. (1963a). Immunological aspects of parasitism. *Nature, London* **197**, 268–269.

Dineen, J. K. (1963b). Antigenic relationship between host and parasite. *Nature, London* **197**, 471–472.

Dipeolu, O. O. and Adam, K. M. G. (1974). On the use of membrane feeding to study the development of *Trypanosoma brucei* in *Glossina*. *Acta Tropica* **31**, 185–201.

Donges, J. (1974). A formula for the mean infection success per miracidium and a method of proving the homogeneous susceptibility of snail populations to trematode infection. *International Journal of Parasitology* **4**, 403–407.

Dorf, M. E., Balner, H., Loes de Groot, M. and Benacerraf, B. (1974). Histocompatibility-linked immune response genes in the rhesus monkey. *Transplantation Proceedings* **6**, 119–123.

Dow, C. and Jarrett, W. F. H. (1960). Age, strain and sex differences in susceptibility to *Cysticercus fasciolaris* in the mouse. *Experimental Parasitology* **10**, 72–74.

Duckett, M. G., Denham, D. A. and Nelson, G. S. (1970). A difference in the longevity and distribution of adult *Trichinella spiralis* in two strains of laboratory mice. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **64**, 25.

Duke, B. O. L., Lewis, D. J. and Moore, P. J. (1966). *Onchocerca-Simulium* complexes. I. Transmission of forest and Sudan-savanna strains of *Onchocerca volvulus*, from Cameroon, by *Simulium damnosum* from various West African bioclimatic zones. *Annals of Tropical Medicine and Parasitology* **60**, 318–336.

Dunham, E. K., Unanue, E. R. and Benacerraf, B. (1972). Antigen binding and capping by lymphocytes of genetic non-responder mice. *Journal of Experimental Medicine* **136**, 403–408.

Dunn, M. C. and Brown, H. W. (1963). Comparison of the susceptibility of CF1, C57 Brown, C3H, C57 Black and DBA 2 strains of mice to *Aspiculuris tetraptera*. *Journal of Parasitology* **49**, (Sect. 2). 32–33.

Ellman, L., Green, I., Martin, W. J. and Benacerraf, B. (1970). Linkage between the Poly-L-lysine gene and the locus controlling the major histocompatibility antigens in strain 2 guinea pigs. *Proceedings of the National Academy of Sciences USA* **66**, 322–328.

Esslinger, J. H. (1962). Behaviour of microfilariae of *Brugia pahangi* in *Anopheles quadrimaculatus*. *American Journal of Tropical Medicine and Hygiene* **11**, 749–758.

Evans, J. V. and Blunt, M. H. (1961). Variation in the gene frequencies of potassium and haemoglobin types in Romney Marsh and Southdown sheep established away from their native environment. *Australian Journal of Biological Science* **14**, 100–108.

Evans, J. V. and Whitlock, J. H. (1964). Genetic relationship between maximum haematocrit values and haemoglobin type in sheep. *Science, New York* **145**, 1318.

Evans, J. V., Blunt, M. H. and Southcott, W. H. (1963). The effects of infection with *Haemonchus contortus* on the sodium and potassium concentrations in the erythrocytes and plasma, in sheep of different haemoglobin types. *Australian Journal of Agricultural Research* **14**, 549–558.

Fadl, A. M. (1971). The susceptibility of London-hooded and Wistar rats to

experimental infection with *Schistosoma mansoni*. *Journal of Helminthology* **45**, 111–116.

Files, V. S. (1951). A study of the vector–parasite relationships in *Schistosoma mansoni*. *Parasitology* **41**, 264–269.

Files, V. S. and Cram, E. B. (1949). A study of the comparative susceptibility of snail vectors to strains of *Schistosoma mansoni*. *Journal of Parasitology* **35**, 555–560.

Fox, E. G. and Schacher, J. F. (1976). A comparison of syngeneic laboratory rat-strains as hosts for *Brugia pahangi*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **70**, 523.

Francis, J. (1966). Resistance of Zebu and other cattle to tick infestation and babesiosis with special reference to Australia: an historical review. *British Veterinary Journal* **122**, 301–307.

Francis, J. and Little, D. A. (1964). Resistance of droughtmaster cattle to tick infestation and babesiosis. *Australian Veterinary Journal* **40**, 247–253.

Friedman, S. B. and Glasgow, L. A. (1973). Interaction of mouse strain and differential housing upon resistance to *Plasmodium berghei*. *Journal of Parasitology* **59**, 851–854.

Gallily, R. and Eliahu, H. (1974). Uptake and degradation of a polypeptide antigen by stimulated and unstimulated macrophages from responder and non-responder mice. *Immunology* **26**, 603–612.

Gagarin, V. G., Dushkin, V. A., Solonenko, I. G. and Zelencov, A. G. (1972). [Infection with *Hydatigera taeniaeformis* strobilocerci in mice of inbred and undetermined strains.] Byulleten Vsesoyuznogo Instituta Gel mintologii im. K. I. Skryabin, No. 8, 17–19. Abstracted in *Helminthological Abstracts* 1974, **43**, 1935.

Garnham, P. C. C. (1964). Factors influencing the development of protozoa in their arthropodan hosts. Second Symposium of the British Society for Parasitology ("Host–Parasite Relationships in Invertebrate Hosts"). pp. 33–50. Blackwell Scientific Publications, Oxford.

Garnham, P. C. C. (1966). "Malaria Parasites and other Haemosporidia". Blackwell Scientific Publications, Oxford.

Garnham, P. C. C. (1970). Primate malaria. *In* "Immunity to Parasitic Animals" (G. J. Jackson, R. Herman and I. Singer, eds), Vol. 2, pp. 767–791. Appleton-Century-Crofts, New York.

Gasser, D. L. (1969). Genetic control of the immune response in mice. I. Segregation data and localization to the fifth linkage group of a gene affecting antibody production. *Journal of Immunology* **103**, 66–70.

Gasser, D. L. and Silvers, W. K. (1974). Genetic determinants of immunological responsiveness. *Advances in Immunology* **18**, 1–66.

Gasser, D. L., Newlin, C. M., Palm, J. and Gonatas, N. K. (1973). Genetic control of susceptibility to experimental allergic encephalomyelitis in rats. *Science New York* **181**, 872–873.

Goble, F. C. (1951). Studies on experimental Chagas' disease in mice in relation to chemotherapeutic testing. *Journal of Parasitology* **37**, 408–414.

Goble, F. C. (1966). Pathogenesis of blood protozoa. *In* "Biology of Parasites" (E. J. L. Soulsby, ed.), pp. 237–254. Academic Press, London and New York.

Goble, F. C. (1970). South American tryponsomes. *In* "Immunity to Parasitic Animals" (G. J. Jackson, R. Herman and I. Singer, eds), Vol. 2, pp. 597–689. Appleton-Century-Crofts, New York.

Gollapudi, V. S. S. and Kind, L. S. (1975). Phenotypic correction of low reagin

production: a genetic defect in the SJL mouse. *Journal of Immunology* **114**, 906–907.

Good, R. A. and Yunis, E. (1974). Association of autoimmunity immunodeficiency in man, rabbits and mice. *Federation Proceedings* **33**, 2040–2050.

Goodman, T. and Koprowski, H. (1962). Study of the mechanism of innate resistance to virus infection. *Journal of Cellular and Comparative Physiology*. **59**, 333–373.

Graham, G. L. and Porter, D. A. (1934). Strains of *Nippostrongylus muris* and their behaviour in various strains of rats. *Journal of Parasitology* **20**, (Suppl.), 323.

Green, I., Paul, W. E. and Benacerraf, B. (1966). The behaviour of hapten-poly-L-lysine conjugates as complete antigens in genetic responder and as haptens in non-responder guinea pigs. *Journal of Experimental Medicine* **123**, 859–879.

Greenberg, J. (1956). Differences in the course of *Plasmodium berghei* infections in some hybrid and backcross mice. *American Journal of Tropical Medicine and Hygiene* **5**, 19–28.

Greenberg, J. and Kendrick, L. P. (1957a). Parasitaemia and survival in inbred strains of mice infected with *Plasmodium berghei. Journal of Parasitology* **43**, 413–419.

Greenberg, J. and Kendrick, L. P. (1957b). Some characteristics of *Plasmodium berghei* passed within inbred strains of mice. *Journal of Parasitology* **43**, 420–427.

Greenberg, J. and Kendrick, L. P. (1958). Parasitaemia and survival in mice infected with *Plasmodium berghei.* Hybrids between Swiss (high parasitemia) and STR (low parasitemia) mice. *Journal of Parasitology* **44**, 492–498.

Greenberg, J. and Kendrick, L. P. (1959). Resistance of malaria in hybrids between Swiss and certain other strains of mice. *Journal of Parasitology* **45**, 263–267.

Greenberg, J., Nadel, E. M. and Coatney, G. R. (1953). The influence of strain, sex and age of mice on infection with *Plasmodium berghei. Journal of Infectious Diseases* **93**, 96–100.

Greenberg, J., Nadel, E. M. and Coatney, G. R. (1954). Differences in survival of several inbred strains of mice and their hybrids infected with *Plasmodium berghei. Journal of Infectious Diseases* **95**, 114–116.

Greenwood, B. M. and Greenwood, A. M. (1971). Malaria infection in adult NZB mice and in adult (NZB × NZW) F_1 hybrid mice. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **65**, 581–585.

Gregory, P. W. (1937). The possibility of establishing within breeds lines of sheep that are genetically resistant to stomach worms. *Proceedings of the American Society for Animal Production* **10**, 316–324.

Gregory, P. W., Miller, R. F. and Stewart, M. A. (1940). An analysis of environmental and genetic factors influencing stomach-worm infestation in sheep. *Journal of Genetics* **39**, 391–400.

Grunbacher, F. J. (1972). Human X chromosome carries quantitative genes for immunoblogulin M. *Science, New York* **176**, 311–312.

Hall, R. D. and Gross, W. B. (1975). Effect of social stress and inherited plasma corticosterone levels in chickens on populations of the northern fowl mite *Ornithonyssus sylviarum. Journal of Parasitology* **61**, 1096–1100.

Hauschka, T. S. (1947). Sex of host as a factor in Chagas' disease. *Journal of Parasitology* **33**, 399–404.

Hawking, F. (1953). Milk diet, P-amino benzoic acid, and malaria (*P. berghei*). *British Medical Journal* **1**, 1201–1202.

Healy, G. R. and Gleason, N. M. (1966). Studies on the pathogenicity of various

strains of *Entamoeba histolytica* after prolonged cultivation, with observations on strain differences in the rats employed. *American Journal of Tropical Medicine and Hygiene* **15**, 294–299.

Hepler, D. I. and Lueker, D. C. (1974). Enhancement of virulence and immunogenicity of *Nematospiroides dubius*. *Journal of Parasitology* **60**, 1057–1058.

Herbert, W. J. and Lumsden, W. H. R. (1967). Differences in the response of mice of two strains to infection with trypanosomes. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **61**, 140–141.

Heremans, J. F. (1974). Immunoglobulin A. *In* "The Antigens" (M. Sela, ed.), Vol. 2, pp. 365–522. Academic Press, London and New York.

Herrick, C. A. (1934). The development of resistance to the protozoan parasite, *Eimeria tenella*, by the chicken. *Journal of Parasitology* **20**, 329–330.

Herzenberg, L. A., McDevitt, H. O. and Herzenberg, L. A. (1968). Genetics of antibodies. *Annual Review of Genetics* **2**, 209–244.

Heyneman, D. (1962). Studies on helminth immunity. I. Comparison between lumenal and tissue phases of infection in the white mouse by *Hymenolepis nana* (Cestoda: Hymenolepididae). *American Journal of Tropical Medicine and Hygiene* **11**, 46–63.

Hildemann, W. H. (1974). Genetics of immune responsiveness. *Annual Review of Genetics* **7**, 19–36.

Honigberg, B. M. (1970.) Trichomonads. *In* "Immunity to Parasitic Animals" (G. J. Jackson, R. Herman and I. Singer, eds), Vol. 2, pp. 469–550. Appleton-Century-Crofts, New York.

Hopkins, C. A. and Stallard, H. E. (1974). Immunity to intestinal tapeworms: the rejection of *Hymenolepis citelli* by mice. *Parasitology* **69**, 63–76.

Horak, I. G., Clark, R. and Gray, R. S. (1968). The pathological physiology of helminth infestations. III. *Trichostrongylus colubriformis*. *Onderstepoort Journal of Veterinary Research* **35**, 195–223.

Howard, J. G., Courtenay, B. M. and Desaymard, C. (1974). Equivalent responsiveness to branched polysaccharides and their dinitrophenyl conjugates in the Biozzi high and low responder lines of mice. *European Journal of Immunology* **4**, 453–457.

Huff, C. G. (1929). The effects of selection upon susceptibility to bird malaria in *Culex pipiens* Linn. *Annals of Tropical Medicine and Parasitology* **23**, 427–442.

Huff, C. G. (1931). The inheritance of natural immunity to *Plasmodium cathemerium* in two species of *Culex*. *Journal of Preventative Medicine* **5**, 249–259.

Hughes, D. L., Harness, E. and Doy, T. G. (1976). The establishment and duration of *Fasciola hepatica* infections in two strains of rats and the development of acquired resistance. *Research in Veterinary Science* **20**, 207–211.

Hynes, H. B. N. and Nicholas, W. L. (1958). The resistance of *Gammarus* spp to infection by *Polymorphus minutus* (Goeze, 1782) (Acanthocephala). *Annals of Tropical Medicine and Parasitology* **52**, 376–383.

Isakovich, J. and Camacaro, A. (1973). [Occurrence of gastrointestinal parasites among goats at the experimental farm for goat production at Loma de Leon Venezuala.] *Barquisimeto, Venezuela: Ministerio de Agricultura y Cria* (*C.I.A. R.C.O.*) *Estacion Experimental et Cuji*. Abstracted in *Helminthology Abstracts* 1975, **44**, 40.

Jackson, G. J., Herman, R. and Singer, I. (Eds) (1970) "Immunity to Parasitic Animals", Vol. 2. Appleton-Century-Crofts, New York.

Jacobs, R. L. (1964). Role of p-amino benzoic acid in *Plasmodium berghei* infection in the mouse. *Experimental Parasitology* **15**, 213–225.

Jarrett, E. E. E. and Urquhart, G. M. (1971). Immunity to nematode infections. *International Review of Tropical Medicine* **4**, 53–96.

Jeffers, T. K. (1968). Quoted by Visco and Burns, 1972.

Jeffers, T. K., Challey, J. R. and McGibbon, W. H. (1970). Response of several lines of fowl and their single-cross progeny to experimental infection with *Eimeria tenella*. *Avian Diseases* **14**, 203–210.

Jenkin, C. R. (1963). Heterophile antigens and their significance in the host parasite relationship. *Advances in Immunology* **3**, 351–376.

Jilek, A. F. and Bradley, R. E. (1969). Hemoglobin types and resistance to *Haemonchus contortus* in sheep. *American Journal of Veterinary Research* **30**, 1773–1778.

Johnson, J. C., Stewart, T. B. and Hale, O. M. (1975). Differential responses of Duroc, Hampshire, and crossbred pigs to a superimposed experimental infection with the intestinal threadworm *Strongyloides ransomi*. *Journal of Parasitology* **61**, 517–524.

Johnston, L. A. Y. (1967). Epidemiology of bovine babesosis in Northern Queensland. *Australian Veterinary Journal* **43**, 427–432.

Kagan, I. G. and Geiger, S. J. (1965). The susceptibility of three strains of *Australorbis glabratus* to *Schistosoma mansoni* from Brazil and Puerto Rico. *Journal of Parasitology* **51**, 622–627.

Kapp, J. A., Pierce, C. W., Schlossman, S. and Benacerraf, B. (1974). Genetic control of immune responses *in vitro*. V. Stimulation of suppressor T cells in non-responder mice by the terpolymer L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10} (GAT). *Journal of Experimental Medicine* **140**, 648–659.

Kartman, L. (1953). Factors influencing infection of the mosquito with *Dirofilaria immitis* (Leidy, 1856). *Experimental Parasitology* **2**, 27–78.

Katiyar, J. C. and Sen, A. B. (1969). Susceptibility of different strains of white rats to *Nippostrongylus brasiliensis* (Travassos, 1914) infection. *Indian Journal of Helminthology* **21**, 81–85.

Katz, D. H. and Benacerraf, B. (1975). The function and interrelationships of T-cell receptors, IR genes and other histocompatibility gene products. *Transplantation Review* **22**, 175–195.

Katz, D. H. and Benacerraf, B. (Eds) (1976). "The Role of Products of the Histocompatibility Gene Complex in Immune Responses". Academic Press, London and New York.

Katz, F. E. and Steward, M. W. (1975). The genetic control of antibody affinity in mice. *Immunology* **29**, 543–548.

Keeling, J. E. D. (1961). Experimental trichuriasis. I. Antagonism between *Trichuris muris* and *Aspiculuris tetraptera* in the albino mouse. *Journal of Parasitology* **47**, 641–646.

Keller, A. E., Leathers, W. S. and Ricks, H. C. (1934). An investigation of the incidence and intensity of infestation of hookworm in Mississippi. *American Journal of Hygiene* **19**, 629–656.

Keller, A. E., Leathers, W. S. and Knox, J. C. (1937). The present status of hookworm infestation in North Carolina. *American Journal of Hygiene* **26**, 437–454.

Kendall, S. B. and Parfitt, J. W. (1959). Studies on the susceptibility of some species of *Lymnaea* to infection with *Fasciola gigantica* and *F. hepatica*. *Annals of Tropical Medicine and Hygiene* **53**, 220–227.

Kennedy, C. R. (1975). "Ecological Animal Parasitology." Blackwell Scientific Publications, Oxford.

Kierszenbaum, F. and Howard, J. G. (1976). Mechanisms of resistance against experimental *Trypanosoma cruzi* infection: the importance of antibodies and

antibody forming capacity in the Biozzi high and low responder mice. *Journal of Immunology* **116**, 1208–1211.

Kilama, W. L. and Craig, G. B. (1969). Monofactorial inheritance of susceptibility to *Plasmodium gallinaceum* in *Aedes aegypti*. *Annals of Tropical Medicine and Parasitology* **63**, 419–432.

Kloosterman, A. (1974). Genetical variation in resistance to *Cooperia oncophora* between calves of the Dutch Friesian breed. *Proceedings of the Third International Congress of Parasitology*, Vol. 2, 734–735.

Kretschmar, W. (1962). Resistenz und Immunität bei mit *Plasmodium berghei* infizierten Mäusen. *Zeitschrift für Tropenmedezin und Parasitologie* **13**, 159–175.

Lackie, A. M. (1975). The activation of infective stages of endoparasites of vertebrates. *Biological Reviews* **50**, 285–323.

Layne, J. N. and Griffo, J. V. (1961). Incidence of *Capillaria hepatica* in populations of the Florida deer mouse *Peromyscus floridanus*. *Journal of Parasitology* **47**, 31–37.

Leathers, W. S., Keller, A. E. and Wyman, B. F. (1936). A state-wide investigation of hookworm in South Carolina. *American Journal of Hygiene* **23**, 600–614.

Leid, R. W. and Williams, J. F. (1974). The immunological response of the rat to infection with *Taenia taeniaeformis*. I. Immunoglobulin classes involved in passive transfer of resistance. *Immunology* **27**, 195–208.

Levine, B. B. and Vaz, N. M. (1970a). Effect of combinations of inbred strain, antigen and antigen dose on immune responsiveness and reagin production in the mouse. A potential mouse model for immune aspects of human atopic allergy. *International Archive of Allergy and Applied Immunology* **39**, 156–171.

Levine, B. B. and Vaz, N. A. (1970b). Two kinds of genetic control of reagin production in the mouse. *Journal of Clinical Investigation* **49**, 58 (abstract).

Levine, B. B., Stember, R. H. and Fotino, M. (1972). Ragweed hay fever: genetic control and linkage to HL-A haplotypes. *Science, New York* **178**, 1201–1203.

Lewert, R. M. and Lee, C. L. (1957). The collagenase enzymes of skin-penetrating helminths. *American Journal of Tropical Medicine and Hygiene* **6**, 473–477.

Lewert, R. M. and Mandlowitz, S. (1963). Innate immunity to *Schistosoma mansoni* relative to the state of connective tissue. *Annals of the New York Academy of Science* **113**, 54–62.

Lilly, F. and Pincus, T. (1973). Genetic control of murine viral leukemogenesis. *Advances in Cancer Research* **17**, 231–277.

Liu, S.-K. (1965). Pathology of *Nematospiroides dubius:* I Primary infections in C3H and Webster mice. *Experimental Parasitology* **17**, 136–147.

Liu, S.-K. (1966). Genetic influence on resistance of mice to *Nematospiroides dubius*. *Experimental Parasitology* **18**, 311–319.

Livingstone, F. B. (1971). Malaria and human polymorphisms. *Annual Review of Genetics* **5**, 33–64.

Long, P. L. (1968). The effect of breed of chickens on resistance to *Eimeria* infections. *British Poultry Science* **9**, 71–78.

Long, P. L. (1970). Some factors affecting the severity of infection with *Eimeria tenella* in chicken embryos. *Parasitology* **60**, 435–447.

Lozner, E. G., Sachs, D. H. and Shearer, G. M. (1974). Genetic control of the antibody response to Staphylococcal nuclease. I IR-NASE: Control of the antibody response to nuclease by the IR region of the mouse H-2 complex. *Journal of Experimental Medicine* **139**, 1204–1214.

Lubinsky, G. (1964). Growth of the vegetatively propagated strain of larval *Echinococcus multilocularis* in some strains of Jackson mice and in their hybrids. *Canadian Journal of Zoology* **42**, 1099–1103.

Lueker, D. C. and Hepler, D. I. (1975). Differences in immunity to *Nematospiroides dubius* in inbred and outbred mice. *Journal of Parasitology* **61**, 158–159.

Lukic, M. L., Cowing, C. and Leskowitz, S. (1975). Strain differences in ease of tolerance induction to bovine γ globulin: dependence on macrophage function. *Journal of Immunology* **114**, 503–506.

Luzzatto, L. (1972). Comments on blood enzyme deficiencies. *Proceedings of the Helminthological Society of Washington* **39**, 100–106.

Luzzatto, L. (1974). Genetic factors in malaria. *Bulletin of the World Health Organization* **50**, 195–202.

Luzzatto, L., Nwachoko-Jarrett, E. S. and Reddy, S. (1970). Increased sickling of parasitized erythrocytes as mechanism of resistance against malaria in the sickle-cell trait. *Lancet* **1**, 319–322.

Macdonald, W. W. (1962a). The selection of a strain of *Aedes aegypti* susceptible to infection with semi-periodic *Brugia malayi*. *Annals of Tropical Medicine and Parasitology* **56**, 368–372.

Macdonald, W. W. (1962b). The genetic basis of susceptibility to infection with semi-periodic *Brugia malayi* in *Aedes aegypti*. *Annals of Tropical Medicine and Parasitology* **56**, 373–382.

Macdonald, W. W. (1963). Further studies on a strain of *Aedes aegypti* susceptible to infection with sub periodic *Brugia malayi*. *Annals of Tropical Medicine and Parasitology* **57**, 452–460.

Macdonald, W. W. (1967). The influence of genetic and other factors on vector susceptibility to parasites. *In* "Genetics of Insect Vectors of Disease" (J. W. Wright and R. Pal, eds), pp. 567–584. Elsevier, Amsterdam.

Macdonald, W. W. (1976). Mosquito genetics in relation to filarial infections. Fourteenth Symposium of the British Society for Parasitology ("Genetic Aspects of Host–Parasite Relationships"). pp. 1–24. Blackwell Scientific Publications, Oxford.

Macdonald, W. W. and Ramachandran, C. P. (1965). The influence of the gene f^m (filarial susceptibility, *Brugia malayi*) on the susceptibility of *Aedes aegypti* to seven strains of *Brugia, Wuchereria* and *Dirofilaria*. *Annals of Tropical Medicine and Parasitology* **59**, 64–73.

Manger, B. R. (1976). Studies on the resistance of mice to *Nematospiroides dubius* Baylis, 1962. *Parasitology* **73**, xiii.

Marcuse, M., Wigand, R. and Piroth, M. (1964). Über die Resistenz von Mäusen gegenüber *Trypanosoma cruzi*. *Zeitschrift für Tropenmedezin und Parasitologie* **15**, 279–288.

Marr, J. S. and Pike, E. H. (1967). The protection of mice by "Corpus Christi" strain of *Trypanosoma cruzi* when challenged with "Brasil" strain. *Journal of Parasitology* **53**, 657–659.

Marsh, D. G. (1975). Allergens and the genetics of allergy. *In* "The Antigens" (M. Sela, ed), Vol. 3, pp. 271–359. Academic Press, London and New York.

Marsh, D. G., Bias, W. B. and Ishizaka, K. (1974). Genetic control of basal serum immunoglobulin E level and its effect on specific reaginic sensitivity. *Proceedings of the National Academy of Sciences USA* **71**, 3588–3592.

Mauel, J. and Behin, R. (1974). Cell mediated and humoral immunity to protozoan infections. *Transplantation Reviews* **19**, 121–146.

McDevitt, H. O. and Benacerraf, B. (1969). Genetic control of specific immune responses. *Advances in Immunology* **11**, 31–74.

McDevitt, H. O. and Chinitz, A. (1969). Genetic control of the antibody response: relationship between immune response and histocompatibility (H-2) type. *Science, New York* **163**, 1207–1208.

McDevitt, H. O. and Landy, M. (Eds) (1972). "Genetic Control of Immune Responsiveness". Academic Press, London and New York.

McDevitt, H. O., Oldstone, M. B. A. and Pincus, T. (1974). Histocompatibility-linked genetic control of specific immune responses to viral infection. *Transplantation Reviews* **19**, 209–225.

McFarlin, D. E., Chung-Ling Hsu, S., Slemenda, S. B., Chou, S. C-H. and Kibler, R. F. (1975). The immune response against an encephalitogenic fragment of guinea pig basic protein in the Lewis and Brown Norway strains of rat. *Journal of Immunology* **115**, 1456–1458.

McGhee, R. B. (1950). The ability of the avian malaria parasite *Plasmodium lophurae*, to infect erythrocytes of distantly related species of animals. *American Journal of Hygiene* **52**, 42–47.

McGhee, R. B. and Sullivan, J. S. (1971). *Plasmodium gallinaceum:* variation in susceptibility of dove erythrocytes. *Experimental Parasitology* **30**, 356–362.

McGreevy, P. B., McClelland, G. A. H. and Lavoipierre, M. M. J. (1974). Inheritance of susceptibility to *Dirofilaria immitis* infection in *Aedes aegypti*. *Annals of Tropical Medicine and Parasitology* **68**, 97–109.

Medina, S., Vas, S. I. and Robson, H. G. (1975). Effect of non specific stimulation on the defence mechanisms of inbred mice. *Journal of Immunology* **114**, 1720–1725.

Micks, D. W. (1949). Investigations on the mosquito transmission of *Plasmodium elongatum* Huff, 1930. *Journal of the National Malaria Society* **8**, 206–218.

Miller, H. M. (1931). Immunity of the albino rat to superinfestation with *Cysticercus fasciolaris*. *Journal of Preventative Medicine* **5**, 453–464.

Miller, L. H., Mason, S. J., Dvorak, J. A., McGinniss, N. H. and Rothman, I. K. (1975). Erythrocyte receptors for (*Plasmodium knowlesi*) malaria: Duffy blood group determinants. *Science, New York* **189**, 561–563.

Miller, M. J., Neel, J. V. and Livingstone, F. B. (1956). Distribution of parasites in the red cells of sickle-cell trait carriers infected with *Plasmodium falciparum*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **50**, 294–296.

Mitchell, G. F., Grumet, F. C. and McDevitt, H. O. (1972). Genetic control of the immune response. The effect of thymectomy on the primary and secondary antibody responses of mice to Poly-L (Tyr, Glu)-, poly-D, L-Ala-, -poly-L-Lys. *Journal of Experimental Medicine* **135**, 126–135.

Moose, J. W. and Williams, J. E. (1963). Susceptibility of *Oncomelania formosana* from three different areas of Taiwan to infection with Formosan strain of *Schistosoma japonicum*. *Journal of Parasitology* **49**, 702–703.

Morgan, A. G. and Soothill, J. F. (1975). Relationship between macrophage clearance of PVP and affinity of anti-protein antibody response in inbred mouse strains. *Nature, London* **254**, 711–712.

Morris, P. J. (1974). Histocompatibility systems, immune response, and disease in Man. *Contemporary Topics in Immunobiology* **3**, 141–169.

Most, H., Nussenweig, R. S., Vanderberg, J., Hermand, R. and Yoeli, M. (1966). Susceptibility of genetically standardized (JAX) mouse strains to sporozoite and blood-induced *Plasmodium berghei* infections. *Military Medicine* **131**, (Suppl), 915–918.

Motulsky, A. G. (1975). Glucose-6-phosphate dehydrogenase and abnormal haemoglobin polymorphism – evidence regarding malarial selection. *In* "The Role of Natural Selection in Human Evolution" (F. M. Salzano, ed.), pp. 271–291. North-Holland Publishing Co., Amsterdam.

Moultrie, F., Edgar, S. A. and King, D. F. (1954). Quoted by Cuckler, 1970.

Mozes, E. (1974). Cellular and molecular analysis of genetic control of the immune

response in mice. *In* "Progress in Immunology II" (L. Brent and J. Holborow, eds), Vol. 2, pp. 191–201. North-Holland Publishing Co., Amsterdam.

Mozes, E. and Fuchs, S. (1974). Linkage between the immune response potential to DNA and the X chromosome. *Nature London* **249**, 167–168.

Mozes, E. and Shearer, G. M. (1972). Genetic control of immune responses. *Current Topics in Microbiology and Immunology* **59**, 168–200.

Mueller, J. F. (1966). The laboratory propagation of *Spirometra mansonoides* (Mueller 1935) as an experimental tool. VII Improved techniques and additional notes on the biology of the parasite (Cestoda). *Journal of Parasitology* **52**, 437–443.

Munoz, J. and Bergman, R. K. (1968). Histamine-sensitizing factors from microbial agents with special reference to *Bordetella pertussis. Bacterial Reviews* **32**, 103–126.

Musoke, A. J. and Williams, J. F. (1975a). Immunoglobulins associated with passive transfer of resistance to *Taenia taeniaeformis* in the mouse. *Immunology* **28**, 97–101.

Musoke, A. J. and Williams, J. F. (1975b). The immunological response of the rat to infection with *Taenia taeniaeformis.* V Sequence of appearance of protective immunoglobulins and the mechanism of action of 7Sγ 2a antibodies. *Immunology* **29**, 855–866.

Nadel, E. M., Greenberg, J., Jay, G. E. and Coatney, G. R. (1955). Backcross studies on the genetics of resistance to malaria in mice. *Genetics*, **40**, 620–626.

Nelson, W. A. (1962). Development in sheep of resistance to the ked *Melophagus ovinus* (L). I. Effects of seasonal manipulation of infections. *Experimental Parasitology* **12**, 41–44.

Nelson, W. A. and Bainborough, A. R. (1963). Development in sheep of resistance to the ked *Melophagus ovinus* (L). III Histopathology of sheep skin as a clue to the nature of resistance. *Experimental Parasitology* **13**, 118–127.

Newton, W. L. (1952). The comparative tissue reaction of two strains of *Australorbis glabratus* to infection with *Schistosoma mansoni. Journal of Parasitology* **38**, 362–366.

Newton, W. L. (1953). The inheritance of susceptibility to infection with *Schistosoma mansoni* in *Australorbis glabratus. Experimental Parasitology* **2**, 242–257.

Newton, W. L., Wright, W. H. and Pratt, I. (1945). Experiments to determine potential mosquito vectors of *Wuchereria bancrofti* in the continental United States. *American Journal of Tropical Medicine* **25**, 253–261.

Noblet, R. and Weathersby, A. B. (1973). *Plasmodium gallinaceum:* effects of various compounds on immunity of susceptible *Aedes aegypti* and refractory *Culex pipiens pipiens. Experimental Parasitology* **34**, 417–425.

Nordin, A. A. and Makinodan, T. (1974). Humoral immunity in aging. *Federation Proceedings* **33**, 2033–2035.

Obiamiwe, B. A. and Macdonald, W. W. (1973). Evidence of a sex-linked recessive gene, *sb,* controlling susceptibility to *B. pahangi. Transactions of the Royal Society of Tropical Medicine and Hygiene* **67**, 32–33.

Ogilvie, B. M. (1971). *Nippostrongylus brasiliensis* in mice: an explanation for the failure to induce worm expulsion from passively immunized animals. *International Journal of Parasitology* **1**, 161–167.

Ogilvie, B. M. (1974). Immunity to parasites (helminths and arthropods). *In* "Progress in Immunology II" (L. Brent and J. Holborow, eds), Vol. 4, pp. 127–135. North-Holland Publishing Co., Amsterdam.

Ogilvie, B. M. and Jones, V. E. (1969). Reaginic antibodies and helminth

infections. *In* "Cellular and Humoral Mechanisms in Anaphylaxis and Allergy" (H. Z. Movat, ed.), pp. 13–22. Karger, Basel.

Ogilvie, B. M. and Jones, V. E. (1973). Immunity in the parasitic relationship between helminths and hosts. *Progress in Allergy* **17**, 93–144.

Oldstone, M. B. A., Dixon, F. J., Mitchell, G. F. and McDevitt, H. O. (1973). Histocompatibility-linked genetic control of disease susceptibility. Murine lymphocytic choriomeningitis virus infection. *Journal of Experimental Medicine* **137**, 1201–1212.

Olivier, L. (1962a). Studies on natural resistance to *Taenia taeniaeformis*. I. Strain differences in susceptibility of rodents. *Journal of Parasitology* **48**, 373–378.

Olivier, L. (1962b). Studies on natural resistance to *Taenia taeniaeformis* in mice. II. The effect of cortisone. *Journal of Parasitology* **48**, 758–762.

Olson, D. and Pimental, D. (1974). Evolution of resistance in a host population to attacking parasite. *Environmental Entomology* **3**, 621–624.

Orihara, M. (1962). Studies on *Cysticercus fasciolaris*, especially on differences of susceptibility among uniform strains of the mouse. *Japanese Journal of Veterinary Research* **10**, 37–55.

Ovary, Z. and Caiazza, S. S. (1975). Further studies on the inheritance of responsiveness to pertussis HSF in mice. *International Archive Allergy and Applied Immunology* **48**, 11–15.

Pal, R. (1967). Genetics of insect vectors of disease. *World Health Organization Chronicle* **21**, 343–350.

Pan, C-T. (1963). Generalized and focal tissue responses in the snail *Australorbis glabratus*, infected with *Schistosoma mansoni*. *Annals New York Academy of Science* **113**, 475–485.

Passwell, J. H., Steward, M. W. and Soothill, J. F. (1974). Intermouse strain differences in macrophage function and its relationship to antibody responses. *Clinical and Experimental Immunology* **17**, 159–167.

Patterson, L. T., Johnson, L. W. and Edgar, S. A. (1961). The comparative resistance of several inbred lines of S. C. white leghorns to certain infectious diseases. *Poultry Science* **40**, 1442.

Pavlovsky, E. N. (1966). "Natural nidality of Transmissible Diseases." University of Illinois Press, Urbana.

Payne, W. J. A. (1970). "Cattle Production in the Tropics" Vol. 1. Longman, London.

Pennoit-De Cooman, E. and De Rycke, P. H. (1970). Experimental secondary echinococcosis of *Echinococcus granulosus*. 1. Development in different strains of mice. *Zeitschrift für Parasit-kunde*. **34**, 362–372.

Perrudet-Badoux, A., Binaghi, R. A. and Biozzi, G. (1975). *Trichinella* infestation in mice genetically selected for high and low antibody production. *Immunology* **29**, 387–390.

Petit, J-P. (1974). Le trypanotolerance. *In* "Les Moyens de Lutte contre les Trypanosomes et leurs Vecteurs". Colloque 12–15 Mars, 1974. pp. 255–256. Institut d'Elevage et de Medecine Veterinaire des Pays tropicaux.

Petty, R. E., Steward, M. W. and Soothill, J. F. (1972). The heterogeneity of antibody affinity in inbred mice and its possible immunopathologic significance. *Clinical and Experimental Immunology* **12**, 231–241.

Pizzi, T., Agosin, M., Christen, R., Huecker, G. and Neghme, A. (1949). Quoted by Goble, F. C. 1970.

Plant, J. and Glynn, A. A. (1974a). Natural resistance to *Salmonella* infection, delayed hypersensitivity and Ir genes in different strains of mice. *Nature, London*, **248**, 345–347.

Plant, J. and Glynn, A. A. (1974b). Reply to D. J. Bradley. *Nature, London*, **250**, 354.

Plant, J. and Glynn, A. A. (1976). Genetics of resistance to infection with *Salmonella typhimurium* in mice. *Journal of Infectious Diseases* **133**, 72–78.

Poinar, G. O. (1969). Arthropod immunity to worms. *In* "Immunity to Parasitic Animals" (G. J. Jackson, R. Herman and I. Singer, eds), Vol. 1, pp. 173–211. Appleton-Century-Crofts, New York.

Radhakrishnan, C. V., Bradley, R. E. and Loggins, P. E. (1972). Host responses of worm-free Florida Native and Rambouillet lambs experimentally infected with *Haemonchus contortus*. *American Journal of Veterinary Research* **33**, 817–823.

Ramakrishnan, S. P., Bruce-Chwatt, L. J., Prakash, S., Chowdbury, D. S., Pattanayak, S., Singh, D. and Dhar, S. K. (1964). Selection of a strain of albino mice refractory to *Plasmodium berghei* infection. *Bulletin of the World Health Organization* **31**, 393–397.

Read, C. P. (1958). Status of behavioural and physiological "resistance". Rice Institute Pamphlet **45**, 36–54.

Reid, W. M. (1955). Comparative resistance of imported standard breeds and native Egyptian strains of poultry to *Ascaridia galli*. *Poultry Science* **34**, 30–35.

Revoltella, R. and Ovary, Z. (1969). Reaginic antibody in different mouse strains. *Immunology* **17**, 45–54.

Richards, C. S. (1970). Genetics of a molluscan vector of schistosomiasis. *Nature, London* **227**, 806–810.

Richards, C. S. (1973). Susceptibility of adult *Biomphalaria glabrata* to *Schistosoma mansoni* infection. *American Journal of Tropical Medicine and Hygiene* **22**, 748–756.

Richards, C. S. (1975). Genetic factors in susceptibility of *Biomphalaria glabrata* for different strains of *Schistosoma mansoni*. *Parasitology* **70**, 231–241.

Richards, C. S. and Merritt, J. W. (1972). Genetic factors in the susceptibility of juvenile *Biomphalaria glabrata* to *Schistosoma mansoni* infection. *American Journal of Tropical Medicine and Hygiene* **21**, 425–434.

Riek, R. F. (1962). Studies on the reactions of animals to infestation with ticks. VI Resistance of cattle to infestation with the tick *Boophilus microplus* (Canestrini). *Australian Journal of Agricultural Research* **13**, 532–550.

Rivera-Ortiz, C-I. and Nussenzweig, R. (1976). *Trichinella spiralis:* anaphylactic antibody formation and susceptibility in strains of inbred mice. *Experimental Parasitology* **39**, 7–17.

Roberts, C. J. and Gray, A. R. (1973). Studies on trypanosome-resistant cattle. II The effect of trypanosomiasis on N'dama, Muturu and Zebu cattle. *Tropical Animal Health Production* **5**, 220–233.

Roberts, J. A. (1968). Acquisition by the host of resistance to the cattle tick, *Boophilus microplus* (Canestrini). *Journal of Parasitology* **54**, 657–662.

Rockefeller Foundation (1950). Quoted by Garnham, 1964.

Rodriguez, P. H. and Craig, G. B. (1973). Susceptibility to *Brugia pahangi* in geographic strains of *Aedes aegypti*. *American Journal of Tropical Medicine and Hygiene* **22**, 53–61.

Rosen, F. S. (1972). Immunological deficiency disease. *In* "Clinical Immunobiology" (F. H. Bach and R. A. Good, eds), Vol. 1, pp. 271–289. Academic Press, London and New York.

Rosenberg, L. T. and Tachibana, D. K. (1969). On mouse complement. Genetic variants. *Journal of Immunology* **103**, 1143–1148.

Rosenberg, M. M., Alicata, J. E. and Palafox, A. L. (1954). Further evidence of

heredity, resistance and susceptibility to cecal coccidiosis in chickens. *Poultry Science* **33**, 972–980.

Ross, J. G. (1970). Genetic differences in the susceptibility of sheep to infection with *Trichostrongylys axei*. A comparison of Scottish Blackface and Dorset breeds. *Researches in Veterinary Science* **11**, 465–468.

Ross, J. G., Lee, R. P. and Armour, J. (1959). Haemonchosis in Nigerian Zebu cattle: the influence of genetical factors in resistance. *Veterinary Record* **71**, 27–31.

Rothwell, T. L. W. (1976). Personal communication.

Rubin, R., Lueker, D. C., Flom, J. O. and Anderson, S. (1971). Immunity against *Nematospiroides dubius* in CFW Swiss Webster mice protected by subcutaneous larval vaccination. *Journal of Parasitology* **57**, 815–817.

Ruitenberg, E. J. and Steerenberg, P. A. (1974). Intestinal phase of *Trichinella spiralis* in congenitally athymic (nude) mice. *Journal of Parasitology* **60**, 1056–1057.

Saoud, M. F. A. (1966). Susceptibility of some planorbid snails to infection with *Schistosoma rodhaini* from Kenya. *Journal of Helminthology* **40**, 379–384.

Scheepers-Biva, M., Bafort, J. and Vincke, I. H. (1967). Untersuchungen sur Resistenzbildung gegen *Plasmodium berghei* bei Mäusen. *Zeitschrift für Tropenmedezin Parasitologie* **18**, 45–48.

Schildt, C. S. and McGibbon, W. H. (1953). Quoted by Cuckler, 1970.

Schmitt-Verhulst, A. M., Mozes, E. and Sela, M. (1974). Genetic control of the immune response to a thymus independent synthetic polypeptide. *Immunogenetics* **1**, 357–369.

Schwartzman, R. M., Rockey, J. H. and Halliwell, R. E. (1971). Canine reaginic antibody. Characterization of the spontaneous anti-ragweed and induced anti-dinitrophenyl reaginic antibodies of the atopic dog. *Clinical and Experimental Immunology* **9**, 549–569.

Scrivner, L. H. (1964a). Breed resistance to ostertagiasis in sheep. *Journal of the American Veterinary Medical Association* **144**, 883–887.

Scrivner, L. H. (1964b). Transmission of resistance to ovine ostertagiasis. *Journal of the American Veterinary Medical Association* **144**, 1024–1027.

Scrivner, L. H. (1967). Genetic resistance to ostertagiasis and haemonchiasis in lambs. *Journal of the American Veterinary Medical Association* **151**, 1443–1446.

Seifert, G. W. (1971). Ecto- and endoparasitic effects on the growth rates of Zebu cross breds and British cattle in the field. *Australian Journal of Agricultural Research* **22**, 839–850.

Selby, G. R. and Wakelin, D. (1973). Transfer of immunity against *Trichuris muris* in the mouse by serum and cells. *International Journal of Parasitology* **3**, 717–722.

Sercarz, E. E., Williamson, A. R. and Fox, C. F. (Eds) (1974). "The Immune System – Genes, Receptors, Signals". Proc. III ICN – UCLA Symposium on Molecular Biology. Academic Press, London and New York.

Sheldon, A. J. (1937). Some experimental studies on *Strongyloides ratti*. *American Journal of Hygiene* **25**, 39–52.

Sher, A. and Cohn, M. (1972). Inheritance of an idiotype associated with the immune response of inbred mice to phosphorylcholine. *European Journal of Immunology* **2**, 319–326.

Sher, A., Mackenzie, P. and Smithers, S. R. (1974). Decreased recovery of invading parasites from the lungs as a parameter of acquired immunity to schistosomiasis in the mouse. *Journal of Infectious Diseases* **130**, 626–633.

Shreffler, D. C. and Cheller, S. D. (1975). The H-2 major histocompatibility complex and the *I* immune response region: genetic variation, function, and organization. *Advances in Immunology* **20**, 125–195.

Silver, D. M. and Lane, D. P. (1975). Dominant non-responsiveness in the induction of autoimmunity to liver-specific F antigen. *Journal of Experimental Medicine* **142**, 1455–1460.

Silver, D. M., McKenzie, I. F. C. and Winn, H. J. (1972). Variations in the responses of C57 BL/10J and A/J mice to sheep red blood cells. I Serological characterization and genetic analysis. *Journal of Experimental Medicine* **136**, 1063–1071.

Smillie, W. G. and Augustine, D. L. (1925). Intensity of hookworm infestation in Alabama. Its relationship to residence, occupation, age, sex and race. *Journal of the American Medical Association* **85**, 1958–1963.

Smith, M. A. and Clegg, J. (1976). Different levels of acquired immunity to *Schistosoma mansoni* in two strains of hamster. *Parasitology* **73**, 47–52.

Smithers, S. R. and Terry, R. J. (1965). Naturally acquired resistance to experimental infections of *Schistosoma mansoni* in the rhesus monkey (*Macaca mulatta*). *Parasitology* **55**, 701–710.

Smithers, S. R. and Terry, R. J. (1967). Resistance to experimental infection with *Schistosoma mansoni* in rhesus monkeys induced by the transfer of adult worms. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **61**, 517–533.

Smithers, S. R. and Terry, R. J. (1969). The immunology of schistosomiasis. *Advances in Parasitology* **7**, 41–93.

Smrkovski, L. L., Larson, C. L. and Songandares-Bernal, F. (1974). Fatal visceral leishmaniasis in a strain of Swiss mice. *Journal of Parasitology* **60**, 718–719.

Smyth, J. D. and Haslewood, G. A. D. (1963). The biochemistry of bile as a factor in determining host specificity in intestinal parasites, with particular reference to *Echinococcus granulosus*. *Annals of the New York Academy of Science* **113**, 234–260.

Snell, G. D. (1968). The H-2 locus of the mouse: observations and speculations concerning its comparative genetics and its polymorphism. *Folia Biologica, Praha* **14**, 335–346.

Soothill, J. F. (1974). Immunity deficiency states. *In* "Clinical Aspects of Immunology" (3rd edition) (R. R. A. Coombs, P. H. G. Gell and P. J. Lachman, eds), pp. 649–687. Blackwell Scientific Publishers, Oxford.

Soothill, J. F., Smith, M. D. and Morgan, A. G. (1975). Immunodeficiency and the allergic aspects of parasitic infection. Thirteenth Symposium of the British Society for Parasitology ("Pathogenic Processes in Parasitic Infection"). pp. 59–68. Blackwell Scientific Publications, Oxford.

Soulsby, E. J. L. (Ed) (1972). "Immunity to Animal Parasites." Academic Press, London and New York.

Sprent, J. F. A. (1959). Parasitism, immunity and evolution. *In* "The Evolution of Living Organisms" (G. S. Leeper, ed.), pp. 149–165. Melbourne University Press, Melbourne, Australia.

Sprent, J. F. A. (1969). Evolutionary aspects of immunity in zooparasitic infections. *In* "Immunity to Parasitic Animals" (G. J. Jackson, R. Herman and I. Singer, eds), Vol. 1, pp. 3–62. North-Holland Publishing Co., Amsterdam.

Spurlock, G. M. (1943). Observations on host–parasite relations between laboratory mice and *Nematospiroides dubius* Baylis. *Journal of Parasitology* **29**, 303–311.

Stewart, M. A., Miller, R. F. and Douglas, J. R. (1937). Resistance of sheep of

different breeds to infestation by *Ostertagia circumcincta*. *Journal of Agricultural Research* **55**, 923–930.

Stiffel, C., Mouton, D., Bouthillier, Y., Heumann, A. M., Decreusefond, C., Mevel, J. C. and Biozzi, G. (1974). Polygenic regulation of general antibody synthesis in the mouse. *In* "Progress in Immunology II" (L. Brent and J. Holborow, eds), Vol. 2, pp. 203–212. North-Holland Publishing Co., Amsterdam.

Stirewalt, M. A. (1956). Penetration of host skin by cercariae of *Schistosoma mansoni*. I. Observed entry into skin of mouse, hamster, rat, monkey and man. *Journal of Parasitology* **42**, 565–580.

Stirewalt, M. A. (1963). Seminar on immunity to parasitic helminths. IV. Schistosome infections. *Experimental Parasitology* **13**, 18–44.

Stirewalt, M. A. (1966). Skin penetration mechanisms of helminths. *In* "Biology of Parasites" (E. J. L. Soulsby, ed.), pp. 41–59. Academic Press, London and New York.

Stirewalt, M. A., Shepperson, J. R. and Lincicome, D. R. (1965). Comparison of penetration and maturation of *Schistosoma mansoni* in four strains of mice. *Parasitology* **55**, 227–235.

Stoll, N. R. (1962). The future of nematology symposium. Biology of nematodes parasitic in animals. *Journal of Parasitology* **48**, 830–838.

Stutman, O. (1974). Cell mediated immunity and aging. *Federation Proceedings* **33**, 2028–2032.

Sucharit, S. and Macdonald, W. W. (1973). *Brugia pahangi* in small laboratory animals: attempts to increase susceptibility of white rats to *Brugia pahangi* by host selection. *S. E. Asian Journal of Tropical Medicine and Public Health* **4**, 71–77.

Taliaferro, W. H. and Stauber, L. A. (1969). Immunology of protozoan infections. *In* "Research in Protozoology" (T-T. Chen, ed.), Vol. 3, pp. 506–564. Pergamon Press, Oxford.

Taylor, A. E. R. (Ed.) (1968). Sixth Symposium of the British Society for Parasitology ("Immunity to Parasites"). Blackwell Scientific Publications, Oxford.

Thomas, H. (1975). Reinfektion und Rezidiv bei *Hymenolepis nana*—Infektion. *Zeitschrift für Parasitenkunde* **46**, 25–33.

Trager, W. (1939). Further observations on acquired immunity to the tick *Dermacentor variabilis*. *Journal of Parasitology* **25**, 137–139.

Trager, W. (1942). A strain of the mosquito *Aedes aegypti* selected for susceptibility to the avian malaria parasite *Plasmodium lophurae*. *Journal of Parasitology* **28**, 457–465.

Tripp, M. R. (1963). Cellular responses of molluscs. *Annals of the New York Academy of Sciences* **113**, 467–474.

Townson, H. (1971). Mortality of various genotypes of the mosquito *Aedes aegypti* following the uptake of microfilariae of *Brugia pahangi*. *Annals of Tropical Medicine and Parasitology* **65**, 93–106.

Turner, J. H. (1959). Experimental strongyloidiasis in sheep and goats. II. Multiple infections: development of acquired resistance. *Journal of Parasitology* **45**, 76–86.

Unanue, E. R. (1972). The regulatory role of macrophages in antigenic stimulation. *Advances in Immunology* **15**, 95–165.

Vachek, H. and Kolsch, E. (1974). The genetic control of T cell mediated immunity. I. Characterization of a mouse strain whose low responsiveness is inherited as a recessive trait. *Immunology* **27**, 507–515.

Valdimarsson, H. (1975). Immunity and immunity deficiency. *In* "The Immune

System: A Course on the Molecular and Cellular Basis of Immunity" (M. J. Hobart and I. McConnell, eds), pp. 317–332. Blackwell Scientific Publications, Oxford.

Vaz, N. M., Phillips-Quagliata, J. M., Levine, B. B. and Vaz, E. M. (1971). H-2-linked genetic control of immune responsiveness to ovalbumin and ovomucoid. *Journal of Experimental Medicine* **134**, 1335–1348.

Visco, R. J. and Burns, W. C. (1972). *Eimeria tenella* in bacteria-free chicks of relatively susceptible strains. *Journal of Parasitology* **58**, 586–588.

Vladutiu, A. O. and Rose, N. R. (1974). HL-A antigens: associations with disease. *Immunogenetics* **1**, 305–328.

Wakelin, D. (1967). Acquired immunity to *Trichuris muris* in the albino laboratory mouse. *Parasitology* **57**, 515–524.

Wakelin, D. (1969). Studies on the immunity of albino mice to *Trichuris muris*. The stimulation of immunity by chemically abbreviated infections. *Parasitology* **59**, 549–555.

Wakelin, D. (1970). The stimulation of immunity and the induction of unresponsiveness to *Trichuris muris* in various strains of laboratory mice. *Zeitschrift für Parasitenkunde*. **35**, 162–168.

Wakelin, D. (1975a). Genetic control of immune responses to parasites: immunity to *Trichuris muris* in inbred and random-bred strains of mice. *Parasitology* **71**, 51–60.

Wakelin, D. (1975b). Immune expulsion of *Trichuris muris* from mice during a primary infection: analysis of the components involved. *Parasitology* **70**, 397–405.

Wakelin, D. (1975c). Genetic control of immune responses to parasites: selection for responsiveness and non-responsiveness to *Trichuris muris* in random-bred mice. *Parasitology* **71**, 377–384.

Wakelin, D. (1975d). Genetic control of immunity to parasites. *Parasitology* **71**, xxv.

Wakelin, D. (1976). Host responses. *In* "Ecological Aspects of Parasitology" (C. R. Kennedy, ed.). pp. 115–141. North-Holland Publishing Co., Amsterdam.

Wakelin, D. and Lloyd, M. (1976). Immunity to primary and challenge infections of *Trichinella spiralis* in mice: a re-examination of conventional parameters. *Parasitology* **72**, 173–182.

Ward, R. A. (1963). Genetic aspects of the susceptibility of mosquitoes to malaria infections. *Experimental Parasitology* **13**, 328–341.

Wardlaw, A. C. (1970). Inheritance of responsiveness to pertussis HSF in mice. *International Archives Allergy and Applied Immunology* **38**, 573–589.

Warwick, B. L., Berry, R. O., Turk, R. D. and Morgan, C. O. (1949). Selection of sheep and goats for resistance to stomachworms, *Haemonchus contortus*. *Journal of Animal Science* **8**, 609–610.

Wassom, D. L., Guss, V. M. and Grundmann, A. W. (1973). Host resistance in a natural host–parasite system. Resistance to *Hymenolepis citelli* by *Peromyscus maniculatus*. *Journal of Parasitology* **59**, 117–121.

Wassom, D. L., DeWitt, C. W. and Grundmann, A. W. (1974). Immunity to *Hymenolepis citelli* by *Peromyscus maniculatus:* genetic control and ecological implications. *Journal of Parasitology* **60**, 47–52.

Watanabe, N., Kojima, S. and Ovary, Z. (1976). Suppression of IgE antibody production in SJL mice. 1 Non specific suppressor T cells. *Journal of Experimental Medicine* **143**, 833–845.

Watson, J. and Riblet, R. (1974). Genetic control of responses to bacterial lipopolysaccharides in mice. I. Evidence for a single gene that influences

mitogenic and immunogenic responses to lipopolysaccharides. *Journal of Experimental Medicine* **140**, 1147–1161.

Watson, J. and Riblet, R. (1975). Genetic control of responses to bacterial lipopolysaccharides in mice. II. A gene that influences a membrane component involved in the activation of bone-marrow-derived lymphocytes by lipopolysaccharides. *Journal of Immunology* **114**, 1462–1468.

Weathersby, A. B. (1960). Further studies on exogenous development of malaria in the haemocoels of mosquitoes. *Experimental Parasitology* **10**, 211–213.

Weathersby, A. B. and McCall, J. W. (1968). The development of *Plasmodium gallinaceum* Brumpt in the haemocoels of refractory *Culex pipiens pipiens* Linn. and susceptible *Aedes aegypti* (Linn.). *Journal of Parasitology* **54**, 1017–1022.

Webster, J. M. (1975). Aspects of the host–parasite relationship of plant parasitic nematodes. *Advances in Parasitology* **13**, 225–250.

Weigert, M., Potter, M. and Sachs, D. (1975). Review: Genetics of the immunoglobulin variable region. *Immunogenetics* **1**, 511–523.

Weinmann, C. J. (1970). Cestodes and acanthocephala. *In* "Immunity to Parasitic Animals" (G. J. Jackson, R. Herman and I. Singer, eds), Vol. 2, pp. 1021–1059. Appleton-Century-Crofts, New York.

Weitz, B. G. F. (1970). Infection and resistance. *In* "The African Trypanosomiases" (H. W. Mulligan, ed.), pp. 97–124. George Allen and Unwin, London.

Wenk, P. (1967). Der Invasionsweg der Metazyklischen larven von *Litomosoides carinii* Chandler 1931 (Filariidae). *Zeitschrift für Parasitenkunde* **28**, 240–263.

Whitlock, J. H. (1955). A study of inheritance of resistance to trichostrongylidosis in sheep. *Cornell Veterinarian* **45**, 422–439.

Whitlock, J. H. (1958). The inheritance of resistance to trichostrongylidosis in sheep. I Demonstration of the validity of the phenomena. *Cornell Veterinarian* **48**, 127–133.

Whitlock, J. H. and Madsen, H. (1958). The inheritance of resistance to trichostrongylidosis in sheep. II Observations on the genetic mechanism in trichostrongylidosis. *Cornell Veterinarian* **48**, 134–145.

Wiener, E. and Bandieri, A. (1974). Differences in antigen handling by peritoneal macrophages from the Biozzi high and low responder lines of mice. *European Journal of Immunology* **4**, 457–463.

Wijers, D. J. B. (1958). Factors that may influence the infection rate of *Glossina palpalis* with *Trypanosoma gambiense*. I. The age of the fly at the time of the infected feed. *Annals of Tropical Medicine and Parasitology* **52**, 385–390.

Wikel, S. K. and Allen, J. R. (1976). Acquired resistance to ticks. I. Passive transfer of resistance. *Immunology* **30**, 311–316.

Wilkinson, P. R. (1955). Observations on infestations of undipped cattle of British breeds with the cattle tick, *Boophilus microplus* (Canestrini). *Australian Journal of Agricultural Research* **6**, 655–665.

Worley, D. E., Meisenhelder, J. E., Sheffield, H. G. and Thompson, P. E. (1962). Experimental studies on *Trichuris muris* in mice with an appraisal of its use for evaluating anthelmintics. *Journal of Parasitology* **48**, 433–437.

Wright, C. A. (1974). Snail susceptibility or trematode infectivity *Journal of Natural History* **8**, 545–548.

Yamashita, J., Ohbayashi, M., Kitamura, Y., Suzuki, K. and Okugi, M. (1958). Studies on echinococcosis. VIII. Experimental *Echinococcosis multilocularis* in various rodents; especially on the difference of susceptibility among uniform strains of the mouse. *Japanese Journal of Veterinary Research* **6**, 135–155.

Young, M. D., Eyles, D. E., Burgess, R. W. and Jeffery, G. M. (1955). Experimental testing of the immunity of negroes to *Plasmodium vivax*. *Journal of Parasitology* **41**, 315–318.

Zelencov, A. G. and Solonenko, I. G. (1973). *Hymenolepis nana* in the seven inbred strains of mice. *In* "Materials of the International Conference on Hymenolepidae". Warszawa, 14–16 Sept., 1973. Warsaw Polish Academy of Sciences Parasitological Committee (1973), 146.

Zuckerman, A. (1968). Basis of host cell-parasite specificity. *In* "Infectious Blood Diseases of Man and Animals. Diseases caused by Protista" (D. Weinman and M. Ristic, eds), pp. 23–36. Academic Press, London and New York.

Notes Added in Proof

A number of papers relevant to this review have appeared since the manuscript was completed in 1976. Those dealing with parasitological aspects will be itemized briefly; the reader is referred to the report of a recent symposium on the genetic regulation of the immune system (Sercarz *et al.*, 1977) for current references dealing with immunological aspects.

Some of the material quoted in the review has now been published more fully, for example, Bradley's work on *L. donovani* in strains of mice (Bradley and Kirkley, 1977; Bradley, 1977), the work of Altaif and Dargie on resistance and susceptibility of sheep to *H. contortus* and *O. circumcincta* (Altaif and Dargie, 1978 a, b, c; Cuperolovic, *et al.*, 1978) and Rothwell's work on selective breeding for responses to *T. colubriformis* in guinea pigs (Rothwell, *et al.*, 1978).

The occurrence and nature of genetically determined resistance to trypanosomiasis in cattle and haemonchosis in sheep was discussed in a number of papers at the 8th International Congress of the World Association for the Advancement of Veterinary Parasitology (see Urquhart, 1978, for a full discussion; Allonby and Preston, 1978; Murray *et al.*, 1978; Preston and Allonby, 1978). Field data on the relationship between haemonchosis and haemoglobin polymorphism in sheep has also been published by Allonby and Urquhart (1976).

Two other papers on genetically-determined variability in response of sheep to nematodes should be mentioned. Al-Sammarrae and Sewell (1977) have described strain variation in relation to *Dictyocaulus filaria*, a species not hitherto considered in such work. Dineen *et al.* (1978) have studied the responses of Merino lambs to vaccination against *T. colubriformis* and shown that, while on the basis of group mean worm recoveries no immunity was stimulated (as previously described), analysis of individual data revealed that vaccinated lambs segregated into "responders", which did show immunity to challenge, and "non-responders", which did not. The worm burdens of the latter were responsible for the high group mean recovery.

An extensive series of papers dealing with both immunological and parasitological aspects of mouse strain variation in response to parasitic infection has been published by Mitchell and his co-workers. The species studied so far have been the protozoans *B. rhodhaini* (Mitchell, 1977) and

P. berghei (Whitelaw, Miller *et al.*, 1977), and the helminths *A. suum* (Mitchell *et al.*, 1976), *N. brasiliensis* (Mitchell *et al.*, 1976) and *T. taeniaeformis* (Mitchell *et al.*, 1977). Other recent papers dealing with strain variation in mice include the identification of a strain highly susceptible to *T. gondii* (Kamei *et al.*, 1976), a study of immunity to *A. suum* in mice with an X-linked, B lymphocyte defect (Brown, Crandall and Crandall, 1977) and a detailed analysis of the development of *N. dubius* (*H. polygyrus*) in a hybrid strain exhibiting greater resistance than either parental strain (Cypess *et al.*, 1977). An interesting corollary of host strain variation in response to infection has emerged from the work of Dobson and Owen (1977), who have shown that repeated passage of *N. dubius* in a particular mouse strain resulted in greater infectivity for that strain, reduced infectivity for another strain, but enhanced immunogenicity in both.

Relatively few papers have appeared which deal with parasites other than protozoa or helminths in vertebrates or with vertebrate hosts other than ruminants and rodents. Kemp *et al.* (1976) have made a detailed study of the survival and growth of the tick *Boophilus microplus* on cattle with varying degrees of resistance, and Mason *et al.* (1977) have presented further data on erythrocyte determinants in relation to infection with *Plasmodium knowlesi.* In contrast, there have been several papers dealing with genetic variation in invertebrate hosts. A number have dealt with the susceptibility and resistance of mosquitoes to infection with filarial nematodes, including *B. pahangi* in *A. aegypti* and *C. pipiens fatigans* (Page and Craig, 1975; Obiamiwe, 1977a, b), *W. bancrofti* in *C. pipiens fatigans* (Zielke and Kuhlow, 1977) and *Waltonella flexicauda* in *A. aegypti* (Terwedow and Craig, 1977). In the latter relationship the factor responsible for susceptibility is known not to be related to that determining susceptibility to *B. pahangi* or *D. immitis.* A further contribution to knowledge of variation in response of the snail *B. glabrata* to *S. mansoni* has been published by Richards (1977).

References

Allonby, E. W. and Preston, J. M. (1978). A comparison of the pathogenesis of *Haemonchus contortus* infections in Red Masai and Merino sheep. *Veterinary Parasitology* (in the press).

Allonby, E. W. and Urquhart, G. M. (1976). A possible relationship between haemonchosis and haemoglobin polymorphism in Merino sheep in Kenya. *Research in Veterinary Science* **20**, 212–214.

Al-Sammarrae, S. A. and Sewell, M. M. H. (1977). The relative susceptibilities of Soay and Blackface sheep to natural infection with *Dictyocaulus filaria. Researches in Veterinary Science* **23**, 383–384.

Altaif, K. I. and Dargie, J. D. (1978a). Genetic resistance to helminths: the influence of breed and haemoglobin type on the response of sheep to primary infections with *Haemonchus contortus. Parasitology* **77** (in the press).

Altaif, K. I. and Dargie, J. D. (1978b). Genetic resistance to helminths: the influence of breed and haemoglobin type on the response of sheep to reinfection with *Haemonchus contortus. Parasitology* **77** (in the press).

Altaif, K. I. and Dargie, J. D. (1978c). Genetic resistance to helminths: a comparison of the development of *Ostertagia circumcincta* infections in Scottish

Blackface sheep of different haemoglobin type. *Research in Veterinary Science* **24**, 391–393.

Bradley, D. J. (1977). Regulation of *Leishmania* populations within the host. II. Genetic control of acute susceptibility of mice to *Leishmania donovani* infection. *Clinical and Experimental Immunology* **30**, 130–140.

Bradley, D. J. and Kirkley, J. (1977). Regulation of *Leishmania* populations within the host. I. The variable course of *Leishmania donovani* infections in mice. *Clinical and Experimental Immunology* **30**, 119–129.

Brown, A. R., Crandall, C. A. and Crandall, R. B. (1977). The immune response and acquired resistance to *Ascaris suum* infection in mice with an X-linked B lymphocyte defect. *Journal of Parasitology* **63**, 950–952.

Cuperlovic K., Altaif, K. I. and Dargie, J. D. (1978). Genetic resistance to helminths: a possible relationship between haemoglobin type and the immune responses of sheep to non-parasitic antigens. *Research in Veterinary Science* **25** (in the press).

Cypess, R. H., Lucia, H. L., Zidian, J. L. and Rivera-Ortiz, C. I. (1977). *Heligmosomoides polygyrus:* temporal, spatial and morphological population characteristics in LAF_1/J mice. *Experimental Parasitology* **42**, 34–43.

Dineen, J. K., Gregg, P. and Lascelles, A. K. (1978). The response of lambs to vaccination at weaning with irradiated *Trichostrongylus colubriformis* larvae: segregation into 'responders' and 'non-responders'. *International Journal of Parasitology* **8**, 59–62.

Dobson, C. and Owen, M. E. (1977). Influence of serial passage on the infectivity and immunogenicity of *Nematospiroides dubius* in mice. *International Journal of Parasitology* **7**, 463–466.

Kamei, K., Sato, K. and Tsunematsu, K. (1976). A strain of mouse highly susceptible to *Toxoplasma. Journal of Parasitology* **62**, 714.

Kemp, D. H., Koudstaal, D., Roberts, J. A. and Kerr, J. D. (1976). *Boophilus microplus:* the effect of host resistance on larval attachments and growth. *Parasitology* **73**, 123–136.

Mason, S. J., Miller, L. H., Shiroishi, T., Dvorak, J. A. and McGinniss, M. H. (1977). The Duffy blood determinants: their role in the susceptibility of human and animal erythrocytes to *Plasmodium knowlesi* malaria. *British Journal of Haematology* **36**, 327–336.

Mitchell, G. F. (1977). Studies on immune responses of parasite antigens in mice. V. Different susceptibilities of hypothymic and intact mice to *Babesia rodhaini. International Archives of Allergy and Applied Immunology* **53**, 385–388.

Mitchell, G. F., Goding, J. W. and Rickard, M. D. (1977). Studies on immune responses to larval cestodes in mice. Increased susceptibility of certain mouse strains and hypothymic mice to *Taenia taeniaeformis* and analysis of passive transfer of resistance with serum. *Australian Journal of Experimental Biological and Medical Sciences* **55**, 165–186.

Mitchell, G. F., Hogarth-Scott, R. S., Edwards, R. D. and Moore, T. (1976). Studies on immune responses to parasite antigens in mice. III. *Nippostrongylus brasiliensis* infections in hypothymic nu/nu mice. *International Archives of Allergy and Applied Immunology* **52**, 95–104.

Mitchell, G. F., Hogarth-Scott, R. S., Edwards, R. D., Lewers, H. M., Cousins, G. and Moore, T. (1976). Studies on immune responses to parasite antigens in mice. I. *Ascaris suum* larvae numbers and responses to phosphorylcholine in infected mice of various strains and in hypothymic nu/nu mice. *International Archives of Allergy and Applied Immunology* **52**, 64–78.

Murray, P. K., Murray, M., Morrison, W. I. and McIntyre, W. I. M. (1978).

Trypanosomiasis in N'dama and Zebu cattle I and II. *Veterinary Parasitology* (in the press).

Obiamiwe, B. A. (1977a). Susceptibility to *Brugia pahangi* of geographical strains of *Culex pipiens fatigans*. *Annals of Tropical Medicine and Parasitology* **71**, 367–370.

Obiamiwe, B. A. (1977b). The fate of ingested *Brugia pahangi* microfilariae in susceptible and refractory strains of *Culex pipiens* and *Aedes aegypti*. *Annals of Tropical Medicine and Parasitology* **71**, 375–377.

Page, C. J. and Craig, G. B. (1975). Variation in filarial susceptibility among East African populations of *Aedes aegypti*. *Journal of Medical Entomology* **12**, 485–493.

Preston, J. M. and Allonby, E. W. (1978). The susceptibility of different breeds of sheep and goats to *Haemonchus contortus* infection in East Africa. *Veterinary Parasitology* (in the press).

Richards, C. S. (1977). *Schistosoma mansoni:* susceptibility reversal with age in the snail host *Biomphalaria glabrata*. *Experimental Parasitology* **42**, 165–168.

Rothwell, T. L. W., Le Jambre, L. F., Adams, D. B. and Love, R. J. (1978). *Trichostrongylus colubriformis* infection of guinea-pigs: genetic basis for variation in susceptibility to infection among outbred animals. *Parasitology* **76**, 201–209.

Sercarz, E. S., Herzenberg, L. A. and Fox, C. F. (Eds) (1977). "Regulatory Genetics of the Immune System" Proc. VI ICN-UCLA Symposia on Molecular and Cellular Biology. Academic Press, London and New York.

Terwedow, H. A. and Craig, G. R. (1977). *Waltonella flexicauda:* development controlled by a genetic factor in *Aedes aegypti*. *Experimental Parasitology* **41**, 272–282.

Urquhart, G. M. (1978). The application of immunity in the control of parasitic disease. *Veterinary Parasitology* (in the press).

Whitelaw, A., Miller, J. F. A. P., Mitchell, G. F., Cox, K. O. and Howard, R. H. (1977). Studies on immune responses to parasite antigens in mice. VI. Delayed type hypersensitivity to blood cells from *Plasmodium berghei*-infected mice. *Cellular Immunology* **32**, 216–222.

Zielke, E. and Kuhlow, F. (1977). Inheritance of susceptibility for infection with *Wuchereria bancrofti* in *Culex pipiens fatigans*. *Tropenmedizin und Parasitologie* **28**, 68–70.

SHORT REVIEWS

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The Regulation of Respiratory Metabolism in Parasitic Helminths

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I. Introduction

The literature on the uptake of carbon dioxide by parasitic helminths and the mechanisms which control carbon flow along respiratory metabolic pathways has recently been reviewed (Bryant, 1975). Unfortunately, an unusually long time elapsed between the completion of the review (early 1973) and its publication (mid 1975). In the interim, a number of important papers have been published which go far in providing answers to questions raised therein. The present work is intended to update the earlier one, and to repair a number of omissions. Abbreviations used in the text are summarized in Table I. Readers unfamiliar with the field may find it helpful to refer to the earlier review.

There, the answer was sought for a number of important questions in biochemical parasitology; why the path leading to succinate and its derivatives is so important in parasite respiration; what role the carbon dioxide incorporation plays in the pathway; how metabolism is regulated at the crucial PEP branch-point in glycolysis. Inevitably, definitive answers were not available and so the questions remain central to the study of respiratory metabolism in helminths. To them should now be added the problems of whether one or more types of mitochondrion, with different functions, are involved in

respiration in a single parasite; whether the TCA cycle has an important role in parasite metabolism; what are the mechanisms controlling mitochondrial permeability and oxidative phosphorylation; and how, during respiration, the redox potentials of the various compartments within parasite cells are maintained. The problems are compounded by the nature of the environment of the helminth, which may present all degrees of anoxia. This brief account does not promise to answer the questions; it assembles and, I hope, critically evaluates some of the data which may be used as a foundation upon which, in due course, the answers will be built.

TABLE 1

Abbreviations used in the text

ATP, ADP, AMP: adenosine tri-, di- and monophosphates
FDPase: fructose-1; 6-diphosphatase (EC 3.1.3.11)
FDP: fructose-1; 6-diphosphate
F6P: fructose-6-phosphate
HK: hexokinase (EC 2.7.1.1)
LDH: lactate dehydrogenase (EC 1.1.1.27)
MDH: malate dehydrogenase (EC 1.1.1.37)
ME: "malic enzyme" (EC 1.1.1.39 and 1.1.1.30)
NAD(P), NAD(P)H: oxidized and reduced nicotinamide adenine dinucleotide (phosphate)
PEP: phosphoenolpyruvate
PEPCK: phosphoenolpyruvate carboxykinase (EC 4.1.32)
PK: pyruvate kinase (EC 2.7.1.40)
PFK: phosphofructokinase (EC 2.7.1.11)
TCA: tricarboxylic acid

A number of reviews on parasite metabolism have been published recently. They include one on bioenergetics in helminths, by Barrett (1976a); two on trematodes by Coles (1973, 1975); a more general one by Kurelec (1975a); and a very complete account, by Pappas and Read (1975), of membrane transport in helminth parasites. I shall endeavour here not to duplicate these works.

II. CESTODES

A. *HYMENOLEPIS DIMINUTA*—THE BIOCHEMISTRY OF THE HOST–PARASITE RELATIONSHIP

Hymenolepis diminuta possesses a mechanism for the mediated uptake of glucose (McCracken and Lumsden, 1975a, b). The mechanisms by which glucose is subsequently oxidized under aerobic and anaerobic conditions have been discussed in the earlier review (Bryant, 1975). The major end products of respiration are succinic, acetic and lactic acids. They are derived not only from glucose absorbed from the medium in which the parasite is bathed, but also from endogenous carbohydrate reserves which are readily utilized or replaced under conditions of starvation or plenty.

The relative proportions of excretory products change as the parasite ages (Watts and Fairbairn, 1974). More succinate is produced, during incubation *in vitro*, by 10 and 14 day old organisms than by 6–7 day old ones. In the younger animals, isozymic forms of LDH are found (Walkey and Fairbairn, 1973), together with isozymic forms of PK (Carter and Fairbairn, unpublished; quoted in Watts and Fairbairn, 1974). Isozymes of these enzymes are less apparent in older specimens of *H. diminuta*, which may be symptomatic of the increasing dependence on the succinate producing pathway.

Watts and Fairbairn (1974) note, from measurements of fresh weights of their samples, that the youngest parasites have considerably greater surface: volume ratios than the oldest ones. Following a suggestion of Fairbairn (1970)—that the excretion of lactic acid by helminths is related to the ease with which strong acids can be excreted when surface:volume ratios are high—they correlate the relative decrease in lactic acid production with increase in this ratio. They point out that the excretion of acetic acid (a weaker acid than lactic) remains constant.

The same authors detected high activities of the pyruvate dehydrogenase complex in *H. diminuta* (77 nmoles acetyl phosphate formed $min^{-1}mg^{-1}$ protein). Excreted acetic acid is therefore most probably produced by the action of this enzyme system, which supports the anaerobic reaction scheme summarized by Bryant (1975).

When levels of adenine nucleotides in the tissue of *H. diminuta* were measured, after freeze-clamping the parasite immediately after its removal from the host, Barrett and Beis (1973a) found that, in spite of the essentially anaerobic metabolism of the parasite, its adenylate energy charge—given by the expression $\frac{[ATP] + \frac{1}{2}[ADP]}{[ATP] + [ADP] + [AMP]}$—is high (0·71).

They also found that about 75% of the NAD in the tissues is present in the oxidized form, whereas about 60% of the NADP occurs in the reduced form. Although Barrett and Beis were unable to determine the proportions of bound and unbound cofactor, nor were they able to distinguish between the contents of different sub-cellular compartments, their results are consistent with the view that NAD is utilized primarily in respiratory metabolism, whereas NADPH is required for synthetic purposes. If this be so, then clearly, mechanisms must exist for reoxidizing NADH under the relatively anaerobic conditions in which *H. diminuta* lives. LDH and MDH undoubtedly assist in this function.

Although it is widely accepted that the intestinal environment is "relatively anaerobic", until recently there has been surprisingly little information about the physical characteristics of the environments occupied by intestinal helminths. During the last three years an important series of papers has been published which provides insight into the physical, chemical and biological relationships which exist between *H. diminuta* and the rat intestine.

The foundation for this work was detailed comparison of the physiologies of normal rat gut and gut carrying an infection of *H. diminuta* (Mettrick, 1971a, b, c, 1972, 1973). Factors investigated included microbial fauna, pH, parasite migration and the competition between parasite and host for

ingested nutrients. The studies established that these factors fluctuate in the normal rat intestine and that, in parasitized animals, the characteristics are very different. In turn, these observations led to a close re-examination of a large number of the other assumptions about the nature of the environment occupied by *H. diminuta* (Mettrick, 1975; Podesta and Mettrick, 1974a, b; 1975; 1976). Included in this work are accounts of the effect of the parasite on pH, gastrointestinal function and, most importantly, on oxygen and carbon dioxide tensions within the rat gut.

The concept that the lumenal contents of the mammalian intestine are anoxic, while the region "close to the mucosa" contains measurable amounts of oxygen is shown to be, to say the least, an oversimplification. Podesta and Mettrick (1974a) found that, in the rat intestine, the aqueous phase of the lumenal contents has an oxygen tension of 40–50 mm Hg. In uninfected intestines, they observed that anoxic conditions may occur in the distal ileum and colon, but that *H. diminuta*, by reducing fluid absorption by the gut, helps to maintain the level of oxygen. Other factors contribute to this phenomenon. The parasitized mucosa decreases in weight, thus reducing the barrier to the diffusion of oxygen across the gut wall. As the parasitized intestine also displays reduced substrate transport and glucose metabolism, less energy is expended within epithelial cells, less oxygen is utilized and pO_2 is effectively raised. pH in the infected intestine is also lower than normal. Since increase in hydrogen ion concentration is accompanied by electron uptake, and since decrease in pH elevates redox potential, then, in the more acidic parasitized intestine, less oxygen is consumed by oxidizing agents in the lumenal contents. Another important effect of depressed pH is to elevate pCO_2.

Mettrick (1975) quotes values for the oxidation–reduction potential (E_h) in uninfected (−195 to −28 mV) and infected (−75 to +76 mV) rats. Generally, the greater the worm biomass, the greater is the increase in E_h. This shift, from strong reducing tendencies to relatively less reduced conditions may provide an explanation for the occurrence of high levels of cytochromes of the b type in intestinal helminths. Such cytochromes have E_h values of about +50 mV (Lehninger, 1970) and are well fitted to act as terminal oxidases under the conditions described by Mettrick (1975). It is possible that here we have the reconciliation of the "aerobic" versus "anaerobic" schools of respiratory metabolism championed most notably by Smith (1969) and Cheah (for example, 1972, 1976a, b) on the one hand and by Saz (for example, 1971, 1972) on the other. This, when taken into account with increased pCO_2 and the capacity of intestinal helminths to utilize CO_2 may mean that, in the words of Podesta and Mettrick (1974a) "the worms, by combining the versatility of CO_2 fixation and aerobic energy metabolism, would appear to be ideally suited for a lumenal existence".

Podesta and Mettrick (1974b, 1975, 1976a) have explored the relationships between transport across the parasite integument and changes in pH. It is beyond the scope of this review to comment on these results in detail, except in so far as they illuminate the process of HCO_3^- absorption in *H. diminuta*.

They showed that the parasite absorbs bicarbonate (it is the main anion accompanying Na^+ absorption) and that a net influx of ions occurs in the

absence of concomitant water influxes. The parasite also secretes hydrogen ions into the gut lumen. Organic acid secretion cannot, of itself, account for the latter observation. The high pCO_2 (250–500 mm Hg) in the parasitized environment, as a consequence of depressed pH, results in the diffusion of CO_2 down its concentration gradient into worm tissue. Within the tissue the equilibrium of the CO_2/HCO_3^- system favours the production of H^+ and HCO_3^-, thus:

$$HCO_3^- + H^+ \rightleftharpoons CO_2 + H_2O$$

The presence of HCO_3^- within the worm leads to buffering problems in which calcareous corpuscles may play a part, and which favours its use in metabolism.

It follows from this that the characteristic metabolic patterns so frequently encountered in helminths—not only parasitic ones—living in so-called anaerobic environments may not be a response to anoxia but to the high, and otherwise toxic levels of CO_2 encountered.

Further support for this hypothesis derives from Mettrick *et al.* (1976) who examined the kinetic behaviour of PEPCK and PK from *H. diminuta*. PEPCK has an acid pH optimum, and is activated by HCO_3^-; PK, however, is inhibited by HCO_3^- and has a low affinity for PEP at acid pH. Accumulation of CO_2 in the environment would thus favour the PEPCK reaction. PEPCK from *M. expansa* has a similarly acidic pH optimum (Behm and Bryant, 1975c) and the suggestion was made that here, too, a CO_2-rich environment might drive the PEPCK reaction. The hypothesis put forward by Mettrick, Podesta and co-workers may well have a very wide application.

The work quoted in this section has shown how, in the establishment of the rat/*H. diminuta* host–parasite relationship, the intestinal environment alters to allow the parasite to compete more favourably with the host. This can be further illustrated. Lesser *et al.* (1975) have shown that maximal rates of glucose transport by *H. diminuta* are maintained over a wider range of pH (5·8–8·5) than is the case for host intestine (6·5–7·5). This may confer an advantage upon the parasite, although the authors remain cautious, both about this and about extrapolating to other parasites. They point out that much higher pH's are encountered in other parasite–intestine systems.

Even with this caveat, there is little doubt that what has been done with *H. diminuta* is a paradigm of what must be done for other parasite–host systems. One can only await eagerly the extension of this type of work to, say, the ruminant gut, as the insight gained by such an approach is of the greatest importance.

B. *MONIEZIA EXPANSA* AND OTHER CESTODES–STUDIES OF METABOLIC REGULATION

The recent work on *H. diminuta* has been directed primarily at an analysis of the interactions between the gut and across the body wall of the parasite. Recent studies on *Moniezia expansa*, however, describe in some detail metabolic events consequent on the uptake of glucose from an artificial medium. Behm and Bryant (1975a) measured the activities of a number of enzymes in

the scolex and anterior portion of this sheep tapeworm. The activities of HK, PFK, FDPase, aldolase, PK and LDH are high in the cytosol fraction only. PEPCK and MDH activities are present in this and the mitochondrial fraction.

Special attention was devoted to the assay of ME, as in *H. diminuta* it occupies a key role in the oxidative pathway. Only very low ME activity was found in the cytosol fraction from *M. expansa* and it was primarily in the carboxylation direction. Traces were found in the mitochondria. On the basis of these results it is not possible to draw any conclusions save that ME plays a very minor role in the oxidative pathway in *M. expansa.*

Determinations of intermediate and end-product concentrations were also made. It was found that considerably more (51%) lactate is produced by *M. expansa* under anaerobic conditions than under aerobic conditions. Succinate production is virtually unaffected. The internal pool size of malate increases during aerobiosis. Mass action ratios were calculated from intermediate concentrations and compared with apparent equilibrium constants. The reactions catalysed by HK, PFK and PK were found to be rate limiting, and thus may regulate the pathway of energy metabolism. The status of the reaction catalysed by PEPCK remains equivocal, as a number of approximations were made in the calculation of the mass action ratio. However, the analysis of PEPCK activity in *H. diminuta* by Mettrick *et al.* (1976) renders it likely that, in *M. expansa* too, the enzyme is regulatory.

In *M. expansa* it was also found that ATP/ADP ratios remain high (1·57–1·67), as do adenylate energy charges (0·70–0·76), irrespective of whether the parasite is maintained under aerobic or anaerobic conditions.

Further studies of three of the potentially regulatory enzymes were made. Earlier work on PK (Bryant, 1972) was extended to include a consideration of the effect of ATP on the enzyme (Behm and Bryant, 1975b). ATP exerts an inhibition (competitive with ADP) on the unactivated enzyme. In the presence of FDP, activated PK is still inhibited by ATP, but the results suggest a mixed type of inhibition.

A partially purified preparation of PFK from *M. expansa* was found to have a pH optimum in the range 7·4–8·0, to be activated by Mg^{2+} or Mn^{2+}, and to exhibit sigmoid kinetics with F6P. ATP decreases the affinity of the enzyme for F6P, an effect partially relieved by F6P, AMP and NH_4^+. The degree of sigmoidicity at pH 8 is markedly different from that observed in enzymes from mammalian sources. Generally, however, PK and PFK can be considered to regulate the pathway in a manner similar to that observed in other organisms.

PEPCK has been partially purified from supernatant and mitochondrial fractions from *M. expansa* (Behm and Bryant, 1975c). The enzymes from the two sources differ. Mitochondrial PEPCK is specifically activated by Mn^{2+}; the cytosolic enzyme is also activated by Mg^{2+}. The forward and back reactions proceed at similar rates, suggesting that the reaction may be readily reversed *in vivo*. Of a number of possible activators and inhibitors, only nucleotide triphosphates were found to have an inhibitory effect. Guanosine diphosphate as substrate permits greater maximal activity than ADP.

A report, by Rasero *et al.* (1968), that there exists in *M. expansa* a pathway from glutamate or α-oxoglutarate to succinate, mediated by succinic semialdehyde dehydrogenase and γ-aminobutyrate:α-oxoglutarate transaminase, was investigated by Cornish and Bryant (1975). They found no evidence to support this contention and concluded that glutamate could be metabolized via a preliminary transamination to a α-oxoglutarate followed by oxidation to succinate.

These studies have been combined in a model which gives a postulated sequence of events which may occur in *M. expansa* scoleces during transition from aerobiosis to anaerobiosis *in vitro* (Bryant and Behm, 1976). Briefly, it suggests that there are two sorts of mitochondrial function. In the first, succinate or NADH may be oxidized aerobically by an electron transfer system using oxygen as the terminal electron acceptor. Malate accumulates within the mitochondria, whence it travels to the cytosol.

The second function is the reduction of fumarate to succinate with the concomitant oxidation of NADH by the fumarate reductase system. The source of fumarate is cytosolic malate. The different functions may be located in the same or different mitochondria, cells or tissues.

If little oxygen is available, the activity of the aerobic system is suppressed and less mitochondrial malate is produced. As the malate pool is also depleted by the production of fumarate for anaerobic respiration, the activity of PK, hitherto inhibited by high concentrations of malate, is enhanced. Lactate production increases. Reducing equivalents to drive the fumarate reductase reaction must originate outside the pathway of energy metabolism (possibly from glutamate oxidation) as the combined LDH and MDH reactions account for all NADH produced earlier in the pathway.

The whole system is finely controlled by relative concentrations of adenine nucleotides. During the transition from aerobiosis to anaerobiosis, aerobic production of ATP falls, derepressing PFK and permitting a greater flow of carbon, thus allowing increased anaerobic production of ATP. Although it is necessary to invoke "transient" changes in concentrations of adenine nucleotides, it is unlikely that they can be measured, as the regulatory mechanisms adjust carbon flow to maintain optimum levels. The adjustments may be seen indirectly as increases in lactate output and decreases in the internal concentration of malate.

There are relatively few studies on the biochemistry of respiration in cestodes other than *H. diminuta* and *M. expansa*. Salminen (1974) investigated electron transfer in adults and plerocercoids of *Diphyllobothrium latum*. Succinate dehydrogenase, as measured by the phenazine methosulphate technique, was found in both stages in similar activities, and K_m values for succinate fall within the range reported for other organisms. Further experiments showed that only adults possess an electron transfer system involving both cytochrome c and cytochrome oxidase. The evidence therefore suggests that adult *D. latum* is capable of complete aerobic oxidation of substrates, whereas the plerocercoid conforms to the widely accepted anaerobic pattern.

Studies on other plerocercoids tend to confirm this. McManus (1975) has shown that PK from the plerocercoid of *Ligula intestinalis* is similar to that

described for other parasites. It shows optimal activity at pH 7·0, is activated by FDP, while ATP and malate inhibit co-operatively. An active PEPCK is also present. The author speculates that, as in *M. expansa*, co-ordinated changes in intracellular concentrations of ATP, malate, FDP and PEP combine to control the activity of PK and thus the major end-products of glucose degradation *in vivo*. Plerocercoids of *Triaenophorus crassus* and *Schistocephalus solidus* also conform to the general anaerobic pattern (Körting, 1976). They, the adults of these species, and the adults of *Bothriocephalus gowkongensis* and *Khawia sinensis* are parasites of fresh water fish and are similar in general metabolism to intestinal parasite of homeotherms.

Köhler and Hanselmann (1974) have carried out a more intensive study on the tetrathyridia larvae of *Mesocestoides corti*. They found that glucose is consumed under aerobic or anaerobic conditions, that lactate and succinate are major end-products, with traces of acetate. Succinate production increases anaerobically, which suggests that it might be a substrate under aerobic conditions. The larva probably has an energy metabolism similar in some respects to that described here for *H. diminuta*, as it has a PK of low activity, regulated by FDP, an active PEPCK, the capacity to decarboxylate malate, and a fumarate reductase. It also possesses all the enzymes necessary for TCA cycle activity, with the probable exception of an NAD-dependent isocitrate dehydrogenase. The authors therefore conclude that the larva is capable of both aerobic and anaerobic metabolism.

III. Nematodes

A. *Ascaris*—aerobic or anaerobic?

Ascaris has been fortunate enough to receive the attentions of biochemists since the 1920s, when Keilin included the parasite in the list of organisms in which he had detected cytochrome. Studies on *Ascaris* have contributed hugely to the understanding of the biochemical basis of parasitism, and it is no accident that some most significant advances in the study of respiration in the last three years have been made with *Ascaris* mitochondria.

Cheah (1974) has refined his methods for isolating *Ascaris* mitochondria, and reports that they contain outer and inner membranes, outer compartments and inner cristal spaces similar to those observed in mammalian mitochondria. Direct estimations of ATP synthesis by the preparations were not made. However, the occurrence of oxidative phosphorylation within them was inferred from the observations that ADP stimulated oxygen uptake and that oligomycin (an inhibitor of electron transport-mediated ATP synthesis) depressed it.

The preparations proved to be only loosely coupled, as oxygen uptake due to succinate or α-glycerophosphate oxidation was not affected by ADP.

There are two points in this study that remain to be clarified. The first is that no transitions between state 3 and state 4 respiration rates were observable, implying either that the technique for isolating mitochondria needs further improvement, or that the analogy between *Ascaris* and mammalian

mitochondria is incomplete. The second is that Barrett and Beis (1973b) were not able to observe α-glycerophosphate oxidation in their studies of glycolysis in *Ascaris*.

Cheah (1974) also assayed cytochrome oxidase in *Ascaris* mitochondrial preparations. He found that ADP stimulates oxygen uptake during ascorbate oxidation, and this is prevented by oligomycin. Once again, the transition from state 3 to state 4 respiration was not clearly observed.

Two further papers by Cheah (1976a, b) suggest the presence of a branched respiratory chain with terminal oxidase activity (cytochromes a_3, a_1 and o). He postulates that the terminal oxidases are controlled by variations in oxygen tension in the small intestine of the host, and that the parasite makes use of

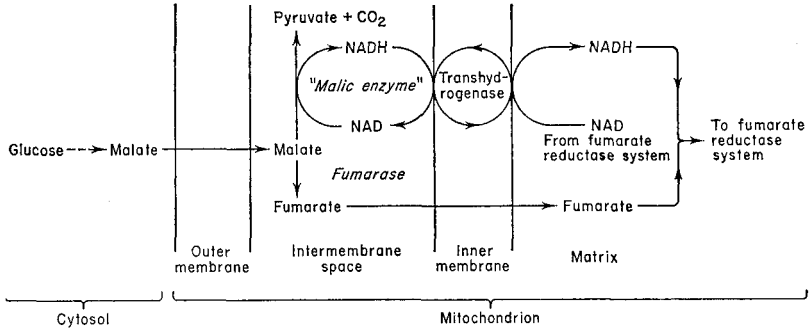

FIG. 1. The oxidation of malate by *Ascaris*, after Rew and Saz (1974).

this cytochrome system *in vivo*, since its components are found to be reduced by the endogenous substrates of the mitochondrial preparations. While accepting the broad hypothesis, it is not yet unequivocal that branched, rather than parallel, cytochrome chains occur. Barrett (1976a) has also expressed the view that branched electron transport systems must be less efficient than straight chain systems, unless they possess a more elaborate regulation mechanism for which there is, as yet, no evidence.

Further support for the anaerobic hypothesis is to be found in Rew and Saz (1974). They used a fractionation procedure, developed for mammalian mitochondria by Sottacasa *et al.* (1967), which enabled them to separate *Ascaris* mitochondria into inner membrane, outer membrane, matrix and intermembrane space fractions. The distribution of enzymes in each fraction conforms to the mammalian pattern *except* that fumarase and NAD-linked "malic" enzyme are found to be associated with the intermembrane space, not with the matrix as in mammals. They thus postulate the metabolic respiratory scheme outlined in Fig. 1, which poses the following problem. If, as in mammals, the inner membrane is impermeable to NADH, and as succinic dehydrogenase is associated with the inner membrane, how then does the energy-yielding reduction of fumarate take place?

The solution appears to reside in the active NADH/NAD transhydrogenase

associated with the inner mitochrondrial membrane of *Ascaris* (Fioravanti and Saz, quoted in Rew and Saz, 1974). The authors suggest that it transports protons from NADH across the inner membrane, resulting in NADH accumulation within the matrix (Fig. 1). Köhler (1976) has achieved a partial purification of the transhydrogenase, has shown that NAD is an acceptor, and that NAD and NADH compete for binding sites on the enzyme. Experiments with inhibitors of NADH:quinone reductase and NADH oxidase support the participation of a quinone system in electron transport, but leave the role of the cytochromes obscure.

There have been two important studies on the mechanisms of oxidative phosphorylation and adenine nucleotide transport in *Ascaris* muscle mitochondria. Van den Bossche (1974) examined the membrane-bound ATP-ase activity (which is generally considered to be a reversal of oxidative phosphorylation) of intact and fragmented mitochondria. He found that intact mitochondria possess little ATP-ase activity, as compared with mammals, and that, although the activity is increased in submitochondrial particles, it still remains relatively low. In addition, the P_i^{32}–ATP exchange reaction is also slow.

In contrast, Beis and Barrett (1974) found that adenine nucleotide exchange in *Ascaris* muscle mitochondria is similar to that found in mammals, as it is specific for ATP and ADP, activated by K^+ and Mg^{2+}, temperature dependent and inhibited by atractyloside.

Barrett (1976b) has also investigated the intermediary metabolism of developing *Ascaris* eggs. The profiles of the enzymes of the respiratory pathways show only quantitative changes during embryonation and the acquisition of infectivity. None of the changes is sufficient to alter the carbon flux through the pathways; dormancy is therefore not associated with these phenomena. Onset and termination of dormancy appear, however, to be associated with changes in the concentrations of some metabolites. Dormant eggs have high ATP/ADP and low cytoplasmic NAD/NADH ratios. Calculations of mass action ratios thus indicate that phosphorylase, HF, PFK and PK are inhibited. The metabolism of the infective egg is apparently intermediate between that of the aerobic developing egg and that of the adult as it possesses a complete TCA cycle, as well as the capacity to fix CO_2 and reduce fumarate.

It is difficult to summarize this section. One may remark that *Ascaris* mitochondria have some notable similarities to mammalian mitochondria (ultrastructure, possession of "mammalian" cytochromes, ATP–ADP exchange) and some notable dissimilarities (ultrastructure, enzyme distribution, ATP-ase activity, possession of "helminthine" cytochromes). The nature of the parasite (aerobic or anaerobic) is still unresolved and will remain so until a detailed study of its environment has been carried out. On the one hand, Bueding, Saz and co-workers (e.g. Kmetec and Bueding, 1961; Saz, 1971, 1972) suggest that respiration is mediated by a fumarate reductase system involving flavoproteins, and is thus fully anaerobic. On the other hand, a second group (e.g. Kikuchi *et al.* 1959; Kikuchi and Ban, 1961; Smith, 1969; Cheah, 1976a) claim that *Ascaris* is aerobic, and that its muscle contains substrate-reducible cytochromes of types a, b and c, and a variety of

terminal oxidases. Cheah (1974) asserts that these observations collectively do not support the anaerobic hypothesis. Nonetheless, they must be considered in conjunction with the observations of the opposing school which show that the parasite does have an anaerobic capacity. It seems likely that both capacities co-exist within the organism, which is thus adapted to possible fluctuations of oxygen tension within its environment and also to high CO_2 concentrations, if they occur. *Ascaris*, it must be remembered, is a whale among nematodes; it is large, probably atypical, and its deeper body tissues may experience different redox conditions from the more superficial tissues. It is unfortunate that the smaller and perhaps more typical nematodes have not been investigated, for obvious reasons, in the same detail.

B. OTHER NEMATODES

A nematode parasite which has received considerable recent attention is the rabbit stomach worm, *Obeliscoides cuniculi* (Hutchinson and Fernando, 1974, 1975). The presence of all the enzymes of glycolysis has been demonstrated, and although variations were observed in developmental stages (early fifth-stage larvae, young and mature adults) they were variations in activities rather than in presence or absence of enzymes. Gluconeogenic capacity is indicated by an active glucose-6-phosphatase and, possibly, pentose phosphate pathway capacity by glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities. All the enzymes of the TCA cycle except α-oxoglutarate dehydrogenase were found, although with commendable caution the authors point out the logical problem involved in establishing the *absence* of a component of a pathway.

Hutchinson and Fernando (1975) therefore suggest that substrate enters the TCA cycle as oxaloacetate produced by PEPCK, the activity of which is much greater than that of PK. The oxaloacetate is rapidly converted to malate by MDH, thus regenerating NAD. A high level of cytosolic fumarase activity and an unusually low level of mitochondrial fumarate reductase activity in the adults lead the authors to suggest that succinate may not be an end-product of metabolism. They note, however, that it is possible that their techniques fail to disrupt the mitochondria, which would result in erroneously low fumarate reductase estimations because the reagents would not have access to the enzyme.

Unlike *Ascaris*, an NADP-dependent malate specific ME was found in the supernatant fraction. The fate of its product, pyruvate, is not clear. With these few reservations, it seems likely that respiratory metabolism in *O. cuniculi* follows the general pattern encountered in other helminths, with, of course, the differences in detail that one has become accustomed to in each genus.

A similarly truncated TCA cycle also seems to be present in *Hyostrongylus rubidus* (Stockdale and Fernando, 1974) and in *Trichinella spiralis* larvae (Boczon, 1974, 1976). Both lack isocitrate dehydrogenase and possess a high MDH activity. In *T. spiralis* the presence of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase implicates the presence of the

pentose phosphate pathway. *T. spiralis* also excretes relatively large amounts of short chain aliphatic amines (Castro *et al.* 1973). However, they are probably derived from the decarboxylation of endogenous amino acids, and the authors suggest that the CO_2 so formed may be utilized in respiratory metabolism at the PEPCK reaction. Thus, if CO_2 levels were to drop during parasitically-induced gut inflammation, the parasite would be able to sustain respiration.

One of the by-products of research on anthelmintics is the information which accrues about parasites which might not otherwise be studied. In two recent papers, Saz and co-workers (Wang and Saz, 1974; Saz and Dunbar, 1975) reported experiments on the respiratory metabolism of three filarial nematodes, *Litomosoides carinii, Dipetalonema viteae* and *Brugia pahangi.* Only *L. carinii* is incapable of sustained motility under anaerobic conditions. Studies of $^{14}CO_2$ and ^{14}C- acetate production from differentially labelled glucose suggest that this nematode has little TCA cycle activity and that, probably, the aerobic requirement of the parasite derives from the single, oxidative conversion of pyruvate to acetate.

D. viteae and *B. pahangi* do not yield acetate; most of the carbon is recovered in glycogen and lactate. There is little TCA cycle activity; Wang and Saz (1974) concluded that the parasites were most probably "homolactate" fermenters.

Saz and Dunbar (1975), in an extension of the above work, examined some of the properties of the PFK's and aldolases from three filariids. They found that the PFK's are similar to those of other helminths (*H. diminuta, Ascaris* and *Schistosoma mansoni*) but differ from mammalian PFK in heightened sensitivity to antimonial drugs. If this result is taken in conjunction with the observation of Behm and Bryant (1975b) that PFK from *M. expansa* displays allosteric properties different from those observed in mammals, it seems possible that in this important regulatory enzyme lies an adaptation common to organisms with low TCA cycle activity.

Ward (1974) studied, *in vitro*, the metabolism of glucose by *Haemonchus contortus.* Chief among his findings is the observation that propan-1-ol is a major excretory product; the author indicates that this need not be considered a radical departure from orthodoxy as the alcohol may be formed by the simple reduction of propionate. Adult *H. contortus* rapidly consumes oxygen; for what purpose it is not yet clear. However, much of the time the parasite was maintained under anaerobic conditions, and like *T. spiralis*, seems capable of utilizing endogenously produced CO_2 in the pathway to propionate.

IV. Trematodes

A. *Fasciola hepatica*—metabolic regulation in a non-intestinal parasite

The evidence which supports the view that there is a general similarity between the pathways of energy metabolism in *Fasciola* and intestinal helminths has already been summarized (Bryant, 1975). Recently, Cheah and Prichard (1975) have studied the electron transport system of *F. hepatica* and,

as in *Ascaris* and *M. expansa*, have found the classical cytochromes of the mammalian type as well as a b-type of cytochrome component containing a CO-reactive "o" cytochrome. Once again, this suggests a dual system which may have evolved in response to an environment of low or fluctuating oxygen tensions and/or high pCO_2.

In *F. hepatica*, the oxidation of carbohydrate leads, *in vitro*, to the production of propionate, acetate, lactate and a little succinate. Wright and Isseroff (1973) have indicated a possible flaw in these observations. They found that the kinetics of acetate absorption in *F. hepatica* indicated that it is an active process, enhanced by pH and inhibited by propionate, butyrate and valerate. They speculate on the role of such a mechanism in an organism which ostensibly produces acetate, and offer the hypothesis that, *in vivo*, acetate production may be low; instead, it is incorporated into higher fatty acids and lipids.

This illustrates yet again the potential gulf between in-vivo and in-vitro experimentation. It is therefore most desirable that extrapolations from experiments *in vitro* to the situation *in vivo* should be made with experimental systems whose validity has in some way been tested. Even then, such extrapolations should be made with caution.

Two attempts have been made to establish and evaluate in-vitro approaches to the study of aspects of metabolism in *F. hepatica*. Hanna and Threadgold (1975) described a method for the preparation of fluke slices and their subsequent maintenance in a completely defined tissue culture medium (M199). Morphological criteria which formed the basis of evaluation included electron microscopical examination of mitochondria, vacuoles and membranous bodies, which retained their structural integrity for up to ten hours. Threadgold and Hanna (1975) extended the criteria of integrity to include oxygen consumption and glucose uptake, the rates for which were rather higher than in intact flukes, although corrections for dry weight tended to abolish the difference. Both processes were inhibited by iodoacetate and accelerated by 2,4-dinitrophenol. The authors concluded that the criteria they had adopted were valid, and that the slices remain in good condition for experimentation for up to 12 hours.

The above-mentioned aspects of morphology and physiology may well be valid criteria of viability but they are not necessarily sufficient to establish metabolic validity. Other criteria could include stability of concentrations of metabolic intermediates, constant rates of excretion of end-products and maintenance of energy status and carbohydrate reserves. Some of these are difficult (if not impossible) to observe *in vivo* but measurements of energy status, as given by adenine nucleotide and glycogen concentrations, and measurements of intermediate levels can be obtained in at least a rough approximation of reality by the modern techniques of freeze-clamping and spectrophotometric assay.

Barret and Beis (1973) give a value of 0·61 for the adenylate energy charge in freshly removed *F. hepatica*. This value is only slightly lower than those encountered in mammalian systems and shows that, in spite of the lower efficiency entailed in the excretion of highly reduced end-products, the parasite

maintains a relatively high energy status. Assays of the nicotinamide nucleotides show that most NAD is in the oxidized state, whereas most NADP is reduced, consistent with the role played by the former in energy metabolism and by the latter in synthetic reactions.

Cornish and Bryant (1976) determined how some of these criteria varied during in-vitro maintenance of the whole animal in a simple balanced salt solution with glucose as a carbon source. The adenylate energy change is 0·62 immediately after removal from the host (which agrees well with the value obtained by Barrett and Beis, 1973) and rises in culture to 0·68 and 0·65 after

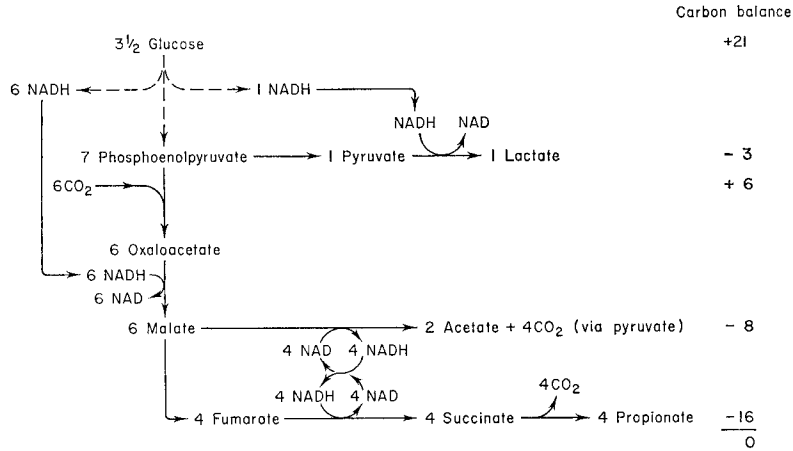

FIG. 2. The oxidation of glucose and the yield of respiratory end-products in *Fasciola hepatica*.

24 and 48 hours, respectively. Glycogen levels remain constant after an initial drop, probably due to expulsion of eggs, and levels of respiratory intermediates are maintained within fairly narrow limits. Output of excretory products approaches linearity after ten hours, and occurs in the remarkably exact proportions of lactate (1·0), acetate (2·02) and propionate (4·05).

This ratio supports the contention of Prichard (1976) and Van Vugt *et al.* (1976) that the acetate producing pathway provides the reducing equivalents to drive the energy yielding fumarate reductase reaction. The relative proportions result from the distribution of carbon, as dictated by the NAD/NADH ratio, at the PEP and malate branch-points in the pathway (Fig. 2).

The pathway illustrated in Fig. 2 is entirely self sufficient in that all NADH produced to the level of PEP is reoxidized by MDH and LDH. Van Vugt *et al.* (1976) have investigated the fate of NADH produced in the acetate branch of the pathway. They observed that, in mitochondria from *F. hepatica*, malate utilization is tightly coupled to phosphorylation as it is ADP dependent, and can be stimulated by the addition of an uncoupler. Determination of phosphorylation efficiencies under anaerobic and aerobic conditions led them to the view that reducing equivalents formed during the oxidation of

malate can be transported either to fumarate or oxygen. In the latter case more ATP is produced, which supports the views of Cheah and Prichard (1975) about electron transport in *F. hepatica*. Under aerobic conditions succinate can act as substrate for the production of ATP.

One of the characteristics of *F. hepatica* is that it produces large quantities of proline, a process which has generated much interest (Ertel and Isseroff, 1974e; Isseroff and Ertel, 1976; Kurelec, 1975a, b). It has been suggested that proline production may be related to the maintenance of redox potential within the cell. Prichard (quoted by Kurelec, 1975a) has criticized this on the basis that the pathway as outlined by Kurelec (1975b) would not result in the oxidation of any more NADH than that produced by the fluke in providing the precursors of the pathway. In any event, the pathway of energy metabolism is, as stated earlier, self-sufficient with respect to the oxidation and reduction of NAD. Kurelec (1975a) argues that only exogenous arginine is necessary to maintain the pathway, the other necessary precursor, pyruvate, being recycled. He also points out that the same pathway occurs in *Schistosoma mansoni*, a trematode which is a so-called "homolactate" fermenter, and which therefore reoxidizes NADH at the LDH level. The significance of the pathway therefore remains to be resolved.

There are few studies on liver fluke developmental stages other than adults. Prichard* (1974) found that the 6-week migratory juveniles were generally similar to adults, but suggested that there might be greater dependence on the TCA cycle. Metzger and Duwel* (1974) followed changes in the activities of a large number of enzymes from 5 weeks to 41 weeks after infection; it appears that, during maturation, the activities of the enzymes of energy metabolism are lowered whereas those of synthetic pathways are increased. This phenomenon seems to be related to the vast egg production of the adult.

B. OTHER TREMATODES AND THE PK/PEPCK BRANCH-POINT

The PEP branch-point in the energy metabolism of parasites has been the object of attention of many biochemical parasitologists. PK/PEPCK ratios are frequently assumed to give a measure of the distribution of carbon along the branches to lactate and succinate. This assumption has been criticized (Bryant, 1975) on the grounds that PK and PEPCK assays are usually conducted at sub-optimal conditions *in vitro* that take no account of the regulatory properties of the enzymes. Further support for this criticism derives from the recent studies of allosterism in PEPCK and PK from *M. expansa* (Behm and Bryant, 1975c), *H. diminuta* (Mettrick *et al.* 1976) and *F. hepatica* (Prichard, 1976).

In the daughter sporocysts of *Microphallus* aerobic oxidation proceeds via glycolysis and the TCA cycle (Marshal *et al.* 1974; McManus and James, 1975a). Anaerobically, succinate, alanine and lactate are produced in the ratio of 2:1:1 (McManus and James, 1975b). The authors suggest that PEPCK and ME are involved and point out the striking similarity between the pattern of excretory products in the parasite and its molluscan host, suggesting that it

* Quoted by Kurelec, 1975a.

is indicative of a long association between the Digenea and the Mollusca. There is circumstantial evidence that PEPCK operates in the direction of CO_2 fixation even under aerobic conditions (McManus and James, 1975c). PK from the daughter sporocysts of *Microphallus similis* (McManus and James 1975d) is strongly activated by FDP and inhibited by ATP; it is apparently not affected by malate.

Köhler and Hanselmann (1973) have investigated the enzyme activities of the pathways of respiratory metabolism in *Dicrocoelium dendriticum* and have concluded that they bear a close similarity to those described in *F. hepatica.* Although all enzymes of the TCA cycle are present, the low activity of α-oxoglutarate dehydrogenase suggests that there is little cycle activity. Pentose phosphate pathway activity is indicated by the detection of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, and the appearance of radiocarbon in CO_2 when the flukes were incubated with 1-^{14}C glucose. CO_2 fixation occurs via PEPCK.

A detailed analysis of the kinetics of PK from *D. dendriticum* shows slight co-operativity between PEP and the enzyme, and that it is strongly activated by low concentrations of FDP (Köhler, 1974). It requires either Mg^{2+} or Mn^{2+} but at the concentrations of ADP prevailing in the tissues, greater reaction velocities are achieved with Mg^{2+}. ATP is inhibitory, especially to the FDP-activated reaction; malate inhibited the reaction only in the absence of FDP. Köhler (1974) therefore suggests a regulatory scheme similar to that postulated for *M. expansa* (Bryant, 1972; Bryant and Behm, 1976), in which, under aerobic conditions, increased concentrations of malate and ATP depress PK and the lactate producing branch.

In *F. hepatica*, Prichard (1976) has shown that PK required Mn^{2+} and K^+ for maximum activity but that in the presence of FDP, Mg^{2+} can substitute for Mn^{2+}. FDP increases the activity of the Mn^{2+} activated enzyme, decreasing the apparent K_m values for PEP and ADP. FDP also relieved the inhibition caused by ATP.

In the same paper, Prichard (1976) reported that NADH stimulated the activity of PEPCK and that, when Mn^{2+} was present, citrate appeared to act synergistically with NADH. Estimates of the concentrations of metabolites suggest that PK would be saturated by in-vivo concentrations of PEP and ADP, whereas PEPCK would not be saturated by PEP. The net effect would therefore be that the enzymes would act to ensure the steady production of pyruvate in accordance with the demands for NADH to generate energy, whilst allowing the respiratory pathways to accommodate carbon flow to the degree of anaerobiosis.

Schistosomes do not appear to have the same problem. They are "homolactate" fermenters; levels of PEPCK are very low, and it is possible that they possess a functional TCA cycle (Coles, 1973a, b). Immature forms are apparently similar to adults.

V. Conclusions

The last three years have seen a number of significant advances in the understanding of respiratory metabolism in helminths. The application of

more sophisticated experimental techniques and of methods of analysis of results is largely responsible. It now appears that the major groups of parasites have more in common than was previously thought; metabolic pathways show generic similarities suggesting that the environments occupied by parasites have a few predominating features in common which dictate the respiratory strategies of the organisms occupying them. Whether the critical factor is oxygen tension or the concentration of carbon dioxide remains to be proved.

The next major advance will no doubt be consequent upon two approaches. The first is the analysis of the distribution of metabolites and enzymes within the cells and tissues of parasites, and also of the transport of molecules across cellular and subcellular barriers. Rew and Saz (1974) have pointed the way with their study on *Ascaris* mitochondria. The second is the analysis of the physical/chemical interactions between environment and parasite, in the manner of Mettrick *et al.* (1976). The extension of all these studies to other parasites and to other life stages is now essential.

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References

Barrett, J. (1976a). Bionergetics in helminths. *In* "The Biochemistry of Parasites and Host-Parasite Relationships" (H. Van den Bossche, ed.). Elsevier North-Holland, Amsterdam.

Barrett, J. (1976b). Intermediary metabolism in *Ascaris* eggs. *In* "The Biochemistry of Parasites and Host–parasite Relationships" (H. Van den Bossche, ed.). Elsevier North-Holland, Amsterdam.

Barrett, J. and Beis, I. (1973a). Nicotinamide and adenosine nucleotide levels in *Ascaris lumbricoides, Hymenolepis diminuta* and *Fasciola hepatica. International Journal for Parasitology* **3**, 271–273.

Barrett, J. and Beis, I. (1973b). Studies on glycolysis in the muscle tissues of *Ascaris lumbricoides* (Nematoda). *Comparative Biochemistry and Physiology* **44B**, 751–761.

Behm, C. A. and Bryant, C. (1975a). Studies of regulatory metabolism in *Moniezia expansa:* general considerations. *International Journal for Parasitology* **5**, 209–217.

Behm, C. A. and Bryant, C. (1975b). Studies of regulatory metabolism in *Moniezia expansa:* the role of phosphofructokinase (with a note on pyruvate kinase). *International Journal for Parasitology* **5**, 339–346.

Behm, C. A. and Bryant, C. (1975c). Studies of regulatory metabolism in *Moniezia expansa:* the role of phosphoenolpyruvate carboxykinase. *International Journal for Parasitology* **5**, 347–354.

Beis, I. and Barrett, J. (1974). Studies on adenine nucleotide exchange in mitochondria from the muscle tissue of *Ascaris lumbricoides* (Nematoda). *International Journal for Parasitology* **4**, 663–666.

Boczon, K. (1974). The tricarboxylic acid cycle and pentose pathway enzymes in *T. spiralis* larvae. *Wiadomosci Parazytologiczne* **20**, 29–39.

Boczon, K. (1976). Effects of some anthelmintics on succinate dehydrogenase-complex in *Trichinella spiralis* larvae. *In* "The Biochemistry of Parasites and Host–Parasite Relationships" (H. Van den Bossche ed.). Elsevier North-Holland, Amsterdam.

Bryant, C. (1972). Metabolic regulation in *Moniezia expansa* (Cestoda): the role pyruvate kinase. *International Journal for Parasitology* **2**, 333–340.

Bryant, C. (1975). Carbon dioxide utilisation, and the regulation of respiratory metabolic pathways in parasitic helminths. *In* "Advances in Parasitology" (Ben Dawes, ed.), Vol. 13, pp. 35–69. Academic Press, New York and London.

Bryant, C. and Behm, C. A. (1976). Regulation of respiratory metabolism in *Moniezia expansa* under aerobic and anaerobic conditions. *In* "The Biochemistry of Parasites and Host–Parasite Relationships" (H. Van den Bossche, ed.). Elsevier North-Holland, Amsterdam.

Castro, G. A., Ferguson, J. D. and Jorden, C. W. (1973). Amine excretion in excysted larvae and adults of *Trichinella spiralis*. *Comparative Biochemistry and Physiology* **45*A***, 819–828.

Cheah, K. S. (1972). Cytochromes in *Ascaris* and *Moniezia*. *In* "Comparative Biochemistry of Parasites" (H. Van den Bossche, ed.), pp. 417–432. Academic Press, London and New York.

Cheah, K. S. (1974). Oxidative phosphorylation in *Ascaris* muscle mitochondria. *Comparative Biochemistry and Physiology* **47*B***, 237–242.

Cheah, K. S. (1976a). Electron transport system of *Ascaris* muscle mitochondria. *In* "The Biochemistry of Parasites and Host-Parasite Relationships" (H. Van den Bossche, ed.). Elsevier North-Holland, Amsterdam.

Cheah, K. S. (1976b). *Ascaris lumbricoides* cytochrome b-560. *In* "The Biochemistry of Parasites and Host–Parasite Relationships" (H. Van den Bossche, ed.). Elsevier North-Holland, Amsterdam.

Cheah, K. S. and Prichard, R. K. (1975). The electron transport systems of *Fasciola hepatica* mitochondria. *International Journal for Parasitology* **5**, 183–186.

Coles, G. C. (1973a). The metabolism of schistosomes: a review. *International Journal of Biochemistry* **4**, 319–337.

Coles, G. C. (1973b). Enzyme levels in cercariae and adult *Schistosoma mansoni*. *International Journal of Parasitology* **3**, 505–510.

Coles, G. C. (1975). Fluke biochemistry–*Fasciola* and *Schistosoma*. *Helminthological Abstracts* **44**, 147–162.

Cornish, R. A. and Bryant, C. (1975). Studies of regulatory metabolism in *Moniezia expansa:* glutamate, and the absence of the γ-aminobutyrate pathway. *International Journal for Parasitology* **5**, 355–362.

Cornish, R. A. and Bryant, C. (1976). The metabolic integrity of *Fasciola hepatica* during *in vitro* maintenance. *International Journal for Parasitology* **6**, 387–392.

Ertel, J. and Isseroff, H. (1974). Proline in fascioliasis: 1. Comparative activities of ornithine-δ-transaminase and proline oxidase in *Fasciola* and in mammalian livers. *Journal for Parasitology* **60**, 574–577.

Fairbairn, D. (1970). Biochemical adaptation and loss of genetic capacity in helminth parasites. *Biological Reviews* **45**, 29–72.

Hanna, R. B. and Threadgold, L. T. (1975). Development of an *in vitro* technique

for cytological investigations of slices of *Fasciola hepatica:* evaluation by morphological criteria. *International Journal for Parasitology* **5**, 321–331.

Hutchinson, G. W. and Fernando, M. A. (1974). Enzymes of gylcolysis and the pentose phosphate pathway during development of the rabbit stomach worm *Obeliscoides cuniculi. International Journal for Parasitology* **4**, 389–395.

Hutchinson, G. W. and Fernando, M. A. (1975). Enzymes of the tricarboxylic acid cycle in *Obeliscoides cuniculi* (Nematoda; Trichostrongylidae) during parasitic development. *International Journal for Parasitology* **5**, 77–82.

Isseroff, H. and Ertel, J. C. (1976). Proline in fascioliasis: III. Activities of pyrroline-5-carboxylic acid reducase and pyrroline-5-carboxylic acid dehydrogenase in *Fasciola. International Journal of Parasitology* **6**, 183–188.

Kikuchi, G. and Ban, S. (1961). Cytochrome in the particulate preparation of the *Ascaris lumbricoides* muscle. *Biochimica Biophysica Acta* **51**, 387–389.

Kikuchi, G., Ramirez, J. and Barron, E. S. G. (1959). Electron transport system in *Ascaris lumbricoides. Biochemica Biophysica Acta* **36**, 335–342.

Kmetec, E. and Bueding, E. (1961). Succinic and reduced diphosphopyridine nucleotide oxidase system of *Ascaris* muscle. *Journal of Biological Chemistry* **236**, 584–591.

Köhler, P. (1974). Metabolic role of pyruvate kinase in the trematode *Dicrocoelium dendriticum. Comparative Biochemistry and Physiology* **49*B***, 335–344.

Köhler, P. (1976). Hydrogen transport in the muscle mitochondria of *Ascaris suum. In* "The Biochemistry of Parasites and Host–Parasite Relationships" (H. Van den Bossche, ed.) Elsevier North-Holland, Amsterdam.

Köhler, P. and Hanselmann, K. (1973). Intermediary metabolism in *Dicrocoelium dendriticum* (Trematoda). *Comparative Biochemistry and Physiology* **45*B***, 825–845.

Köhler, P. and Hanselmann, K. (1974). Anaerobic and aerobic energy metabolism in the larvae (Tetrathyridia) of *Mesocestoides corti. Experimental Parasitology* **36**, 178–188.

Körting, W. (1976). Metabolism in parasitic helminths of freshwater fish. *In* "The Biochemistry of Parasites and Host-Parasite Relationships" (H. Van den Bossche ed.). Elsevier North-Holland, Amsterdam.

Kurelec, B. (1975a). Molecular biology of helminth parasites. *International Journal of Biochemistry* **6**, 375–386.

Kurelec, B. (1975b). Catabolic path of arginine and NAD regeneration in the parasite *Fasciola hepatica. Comparative Biochemistry and Physiology* **51*B***, 151–156.

Lehninger, A. L. (1970). "*Biochemistry.*" 1st edition p. 368. Worth Publishers Inc., New York.

Lesser, R. D., McCracken, R. O. and Lumsden, R. D. (1975). The effect of ambient pH changes on glucose absorption by the tapeworm *Hymenolepis diminuta. Comparative Biochemistry and Physiology* **52*A***, 97–100.

Marshal, I., McManus, D. P. and James, B. L. (1974). Glycolysis in the digestive gland of healthy and parasitised *Littorina saxatilis rudis* (Maton) and in the daughter sporocysts of *Microphallus similis* (Jäg.) *Comparative Biochemistry and Physiology* **49*B***, 291–299.

McCracken, R. O. and Lumsden, R. D. (1975a). Structure and function of parasite surface membranes – I. Mechanism of phlorizin inhibition of hexose transport by the cestode *Hymenolepis diminuta. Comparative Biochemistry and Physiology* **50*B***, 153–157.

McCracken, R. O. and Lumsden, R. D. (1975b). Structure and function of parasite surface membranes – II. Concanavalin A absorption by the cestode

Hymenolepis diminuta and its effect on transport. *Comparative Biochemistry and Physiology* **52B**, 331–337.

McManus, D. P. (1975). Pyruvate kinase in the plerocercoid of *Ligula intestinalis* (Cestoda:Pseudophyllidea). *International Journal of Biochemistry* **6**, 79–84.

McManus, D. P. and James, B. L. (1975a). Tricarboxylic acid cycle enzymes in the digestive gland of *Littorina saxatilis rudis* (Maton) and the daughter sporocysts of *Microphallus similis* (Jäg.) (Digenea:Microphallidae). *Comparative Biochemistry and Physiology* **50B**, 491–495.

McManus, D. P. and James, B. L. (1975b). Anaerobic glucose metabolism in the digestive gland of *Littorina saxatilis rudis* (Maton) and in the daughter sporocysts of *Microphallus similis* (Jäg.) (Digenea:Microphallidae). *Comparative Biochemistry and Physiology* **51B**, 293–297.

McManus, D. P. and James, B. L. (1975c). The aerobic metabolism of ^{14}C-sugars and $^{14}CO_2$ by the daughter sporocysts of *Microphallus similis* (Jäg.) and *Microphallus pygamaes* (Levinsen) (Digenea: Microphallidae). *International Journal for Parasitology* **5**, 177–182.

McManus, D. P. and James, B. L. (1975d). Pyruvate kinases and carbon dioxide fixating enzymes in the digestive gland of *Littorina saxatilis rudis* (Maton) and in the daughter sporocysts of *Microphallus similis* (Jäg.) *Comparative Biochemistry and Physiology* **51B**, 299–306.

Mettrick, D. F. (1971a). *Hymenolepis diminuta:* pH changes in rat intestinal contents and worm migrations. *Experimental Parasitology* **29**, 386–401.

Mettrick, D. F. (1971b). Effect of host dietary constituents on intestinal pH and the migrational behaviour of the rat-tapeworm, *Hymenolepis diminuta. Canadian Journal of Zoology* **49**, 1513–1525.

Mettrick, D. F. (1971c). The microbial fauna, nutritional gradients and physicochemical characteristics of the small intestine of uninfected and parasitized rats. *Canadian Journal of Physiology and Pharmacology* **49**, 972–984.

Mettrick, D. F. (1972). Changes in the distribution and chemical composition of *Hymenolepis diminuta*, and the intestinal nutritional gradients of uninfected and parasitized rats following a glucose meal. *Journal of Helminthology* **46**, 407–429.

Mettrick, D. F. (1973). Competition for ingested nutrients between the tapeworm *Hymenolepis diminuta* and the rat host. *Canadian Journal of Public Health* **64**, 70–82.

Mettrick, D. F. (1975). *Hymenolepis diminuta:* effect on the oxidation-reduction potential in the mammalian gastrointestinal canal. *Experimental Parasitology* **37**, 223–232.

Mettrick, D. F., Mustafa, T., Podesta, R. B., Moon, T. W. and Hulbert, W. (1976). Anaerobes in an aerobic environment: role of CO_2 in energy metabolism in *Hymenolepis diminuta. In* "The Biochemistry of Parasites and Host-Parasite Relationships" (H. Van den Bossche, ed.). Elsevier North-Holland Amsterdam.

Pappas, P. W. and Read, C. P. (1975). Membrane transport in helminth parasites: a review. *Experimental Parasitology* **37**, 469–530.

Podesta, R. B. and Mettrick, D. F. (1974a). Pathophysiology of cestode infections: effect of *Hymenolepis diminuta* on oxygen tensions, pH and gastrointestinal function. *International Journal for Parasitology* **4**, 277–292.

Podesta, R. B. and Mettrick, D. F. (1974b). Components of glucose transport in the host-parasite system, *Hymenolepis dimunita* (Cestoda) and rat intestine. *Canadian Journal of Physiology and Pharmacology* **52**, 183–197.

Podesta, R. B. and Mettrick, D. F. (1975). *Hymenolepis diminuta:* acidification and bicarbonate absorption in the rat intestine. *Experimental Parasitology* **37**, 1–14.

Podesta, R. B. and Mettrick, D. F. (1976). The inter-relationships between the *in situ* fluxes of water, electrolytes and glucose by *Hymenolepis diminuta*. *International Journal for Parasitology* **6**, 163–172.

Prichard, R. K. (1976). Regulation of pyruvate kinase and phosphoenolpyruvate carboxykinase activity in adult *Fasciola hepatica* (Trematoda). *International Journal for Parasitology* **6**, 227–233.

Rasero, F. S., Monteoliva, M. and Mayor, F. (1968). Enzymes related to 4-aminobutyrate metabolism in intestinal parasites. *Comparative Biochemistry and Physiology* **25**, 693–701.

Rew, R. S. and Saz, H. J. (1974). Enzyme localization in the anaerobic mitochondria of *Ascaris lumbricoides*. *Journal of Cellular Biology* **63**, 125–135.

Salminen, K. (1974). Succinate dehydrogenase and cytochrome oxidase in adult and plerocercoid *Diphyllobothrium latum*. *Comparative Biochemistry and Physiology* **49B**, 87–92.

Saz, H. J. (1971). Facultative anaerobiosis in the invertebrates: pathways and control systems. *American Zoologist* **11**, 125–135.

Saz, H. J. (1972). Comparative Biochemistry of carbohydrates in nematodes and cestodes. *In* "The Comparative Biochemistry of Parasites". (H. Van den Bossche, ed.), pp. 33–47. Academic Press, New York and London.

Saz, H. J. and Dunbar, C. A. (1975). The effects of stibophen on phosphofructokinases and aldolases of adult filariids. *Journal of Parasitology* **61**, 794–801.

Smith, M. H. (1969). Do intestinal parasites require oxygen? *Nature London* **223**, 1129–1132.

Sottocasa, G. L., Kuylenstierna, B., Ernster, L. and Bergstrand, A. (1967). An electron-transport system associated with the outer membrane of liver mitochondria. A biochemical and morphological study. *Journal of Cellular Biology* **32**, 415–438.

Stockdale, P. H. G. and Fernando, M. A. (1974). Some enzymes associated with the metabolism of phosphoenolpyruvate in *Hyostrongylus rubidus*. *Journal of Parasitology* **60**, 178.

Threadgold, L. T. and Hanna, B. (1975). Development of an *in vitro* technique for cytological investigations of slices of *Fasciola hepatica:* evaluation by physiological criteria. *International Journal of Parasitology* **5**, 333–337.

Van den Bossche, H. (1974). Adenosine triphosphatase activity in *Ascaris* muscle mitochondria. *Comparative Biochemistry and Physiology* **49B**, 71–78.

Van Vugt, F., Kalaycioglu, L. and Van den Bergh, S. G. (1976). ATP production in *Fasciola hepatica* mitochondria. *In* "The Biochemistry of Parasites and Host–Parasite Relationships" (H. Van den Bossche, ed.). Elsevier North-Holland, Amsterdam.

Walkey, M. and Fairbairn, D. (1973). L-(+)-lactate dehydrogenase from *Hymenolepis diminuta* (Cestoda). *Journal of Experimental Zoology* **183**, 365–374.

Wang, E. J. and Saz, H. J. (1974). Comparative biochemical studies of *Litomosoides carinii, Dipetalonema viteae,* and *Brugia pahangi* adults. *Journal of Parasitology* **60**, 316–321.

Ward, P. F. V. (1974). The metabolism of glucose by *Haemonchus contortus, in vitro*. *Parasitology* **69**, 175–190.

Watts, S. D. M. and Fairbairn, D. (1974). Anaerobic excretion of fermentation acids by *Hymenolepis diminuta* during development in the definitive host. *Journal of Parasitology* **60**, 621–625.

Wright, R. W. and Isseroff, H. (1973). Further studies on the absorption of acetate by *Fasciola hepatica*. *Comparative Biochemistry and Physiology* **45B**, 95–99.

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Industrial Development and Field Use of the Canine Hookworm Vaccine

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I. Introduction

In a previous article (Miller, 1971) recent advances in knowledge of the host–parasite relationship in hookworm infection of dogs were reviewed. Much of the newer information in that review was generated as a by-product from the earlier phases of a rather intensive and extensive project, the primary aim of which was to investigate the possibilities of vaccinating dogs against diseases caused by hookworms. Since preparation and publication of that review, a vaccine* containing gamma-irradiated third stage larvae of *Ancylostoma caninum* has been perfected, tested, licensed, manufactured, introduced for sale and then later discontinued. The purpose of this brief article is to review the more significant aspects of the research and development projects that facilitated licensing, production and introduction of the vaccine in 1973, field experiences in its use by approximately 1500 veterinarians in practice in almost every state of the continental United States of America, and the reasons for suspension of manufacture and hence sale in 1975.

II. Improvements

A. Culture

At the time the research project was transferred from the Wellcome Laboratories for Experimental Parasitology at the University of Glasgow to

* Canine Hookworm Vaccine, Jensen-Salsbery Laboratories.

Jensen-Salsbery Laboratories in late 1969, much of the data and information presented and discussed in the earlier report (Miller, 1971) had been established. Work at Glasgow in 1967–69 had shown that it was possible to produce large numbers of infective larvae by the "dirty" traditional methods of culture. In these methods faecal material from infected dogs was spread on to, or mixed into some vehicle (e.g. paper, sphagnum moss, charcoal, Vermiculite) from which the infective larvae were subsequently harvested and concentrated by washing, filtration, sedimentation and decantation. Larvae produced by these traditional methods were found, when adjusted to a concentration of about 1000 per ml, to be contaminated with at least 10^6 to 10^8 micro-organisms per ml. Micro-organisms identified included coliforms and other enteric bacteria (e.g. *Escherichia coli, Proteus* spp., *Streptococci* and *Pseudomonas*), environmental aerobes (largely *Bacillus* species), anaerobes (*Clostridia*) and numerous other unidentified organisms including fungi and yeasts. Inoculation of such heavily contaminated larval preparations, since subcutaneous inoculation had been shown to be the method of choice for stimulation of maximum and uniform resistance (Miller, 1965a), resulted in a surprisingly low (5–10%) but still practically unacceptable incidence of abscesses and cellulitis. Additionally the United States Department of Agriculture regulations concerning sterility or cleanliness of injectible veterinary vaccines that prevailed in the 1960s permitted no more than 100 viable micro-organisms per dose, a value far below the level in larvae soon after harvest from faecal cultures.

Various processes and substances (washing and "Baermanization" with disinfectants and chemical sterilents) were investigated with the aim of cleaning the larval suspension. It was found that lowering the temperature of the larval suspension to 4–6°C and exposing the larvae to low concentrations (0·1–0·5%) of formalin in phosphate-buffered saline contained in shallow containers (not more than 1·0 cm depth) helped in cleaning the preparation. The clean larval preparation was then filtered and washed several times with tris-buffered saline containing antibiotic bacteriastats and fungistats. The microbial count in the final larval preparation, determined both by dilution and plating, and also by dilution to endpoint in standard liquid test media (fluid thioglycollate at 37°C, soybean casein digest medium at 37°C and 25°C), was reduced to levels compatible with the 1960s requirements. Counts as low as 5–50 bacteria and/or fungi per single dog dose were achieved. It was then found that clean larvae had a much longer shelf life since they remained motile and infective for six months. Larvae in the "dirty" preparations survived only for 2–4 weeks before becoming overgrown and dying. It was also found, as might have been expected, that the incidence of adverse local reactions following inoculation of this clean material was reduced to less than 1 per 1000, a value not uncommonly associated with inoculation of sterile parenteral products and antiobiotics, when skin contaminants may be carried into the tissues by the needle. This clean vaccine was also shown to be highly effective under simulated field trial conditions (Miller *et al.*, 1970).

Unfortunately, as it seemed at that time, the U.S.D.A. regulation defining sterility changed again in the late 1960s. Within the context of these newer

requirements even the "clean" vaccine was unsatisfactory. The last few organisms could not be removed by formalin washing processes. Residual organisms that were isolated in the "clean" vaccine included a few *Bacillus* spp., a rare *Clostridium* and occasional yeasts. For practical purposes subcutaneous inoculation of such small numbers of organisms did not present any significant problems. The results of experiments with cultures of organisms that were isolated from such vaccines showed that numbers many times (10^3+) greater than present in the single dose of "clean" vaccine could be safely inoculated subcutaneously (and intramuscularly) to dogs without any local or systemic reactions. On a basis that included exhaustive microbiological and safety tests to identify any contaminant organisms and to prove their safety, the vaccine came close to receiving U.S.D.A. clearance for a field trial in 1971. The microbiological quality control requirements and potential ramifications were, however, horrendous. In retrospect, it is difficult to see how a commercial product could have survived under these conditions.

At this point and with the assistance of Professor P. P. Weinstein of the University of Notre Dame, studies of methods of axenic cultivation of hookworm larvae were commenced. In approximately one year a sterile culture technique on an industrial scale was perfected at Jensen-Salsbery Laboratories. The procedure started with harvesting, washing and sterilization of hookworm eggs from the faeces of infected dogs by filtration, centrifugation and chemical treatment. The culture medium was based on, and contained some of the ingredients of media described by Bolla *et al.* (1972) and Hansen and Berntzen (1969). The medium included *E. coli*, haemoglobin, cholesterol and several inorganic chemicals. After five days incubation at 25°C, larvae were harvested from the medium by filtration and resuspended in phosphate-buffered saline containing antibiotic preservatives (lincomycin hydrochloride and gentamicin sulphate) that remain stable in liquid preparations. After tests for sterility of each culture lot, lots were batched and irradiated. Tests were conducted on each batch to measure attenuation, by infection of dogs and subsequent enumeration of their intestinal worm burdens. The numbers of sterile female worms recovered from these dogs were compared with numbers of worms from control dogs inoculated with normal larvae of the same batch. Satisfactory sterile irradiated batches were then combined in proportions calculated to give mean infectivity values (count of sterile female worms at eight days after inoculation) within the specified range. The upper limit of this range had been determined previously as that which, in conjunction with the number of larvae per single dog dose, allowed an adequate margin of safety in three-week-old pups as small as 1 lb. Formulation was also adjusted such that the vaccine would still stimulate adequate immunity in any dog at the end of label shelf life. The reader will appreciate that for commercial reasons, the exact details of cultivation, harvest, irradiation, attenuation values and larval count per dose are not provided. By mid-1972 the new process had been adequately tested so that sufficient sterile vaccine was available and permission was granted for field trials in Georgia, South Carolina, Kansas and Missouri to be conducted by veterinarians in practice utilizing dogs owned by the public. The results of these trials, in which 848 dogs of

numerous breeds, from four weeks to six years old, were given approximately 1600 inoculations, did not provide any indications of potential safety problems.

B. IRRADIATION

While in the earlier work larvae were attenuated by exposure to X-rays this process is less convenient, is subject to greater potential calibration errors, and is much more time consuming than attenuation with gamma radiation from radioisotopes (e.g. cobalt-60), especially when the latter is effected in industrial irradiators. Physical conditions could also be more easily controlled.

Tests were conducted to compare the effects of X-rays and gamma rays and to investigate the effect on attenuation of various physical factors during the irradiation process. It was found that the effects of X-rays and gamma rays were, for all practical purposes, identical provided that the larval batch and all physical factors were the same. Physical factors that were shown to exert an influence on radiation attenuation include temperature (both before and during irradiation) and concentration of larvae. Indeed it was found that these two factors, especially when changed in conjunction, could produce as great a variation in radiation-attenuation (measured by worm burdens in dogs necropsied at a set interval post-inoculation) as could a change in radiation dosage of as much as 130 krad. For instance, a change in temperature from 25°C to 10°C, and a change in concentration from 2500 larvae per ml to 125000 larvae per ml could induce the same attenuation changes as could be achieved by a radiation dose change from 20000 to 120000 rads. A temperature change of as little as 2–3°C during irradiation of the larval suspension could have surprisingly large effects. The rate of delivery of the radiation was not important, provided that the physical constants did not change during the entire radiation time and for the half hour before starting irradiation. Due note should be taken by others working in the field of radiation-attenuation of parasites. Unexpected and otherwise inexplicable variations between different investigators and between successive experiments by the same investigator using the same equipment, may be attributable to such factors.

C. SHELF LIFE

Reference has already been made to the improvements in shelf life achieved by a change from "dirty" larvae, not subject to extensive washing and cleaning processes, to larvae that have been surface sterilized by exposure to formalin. Further improvements in the culture process, that achieved sterility in the final product and produced more uniformly fed and developed larvae, also conferred an additional bonus in terms of shelf life. Maximum larval longevity in cleaned and chemically-treated faecal-origin, larvae approximated six months. A label shelf life of six months from the date of harvest, however, means a much less useful practical (commercial) shelf life. Larvae harvested from the sterile culture process survived for 12–14 months from date of harvest. A useful shelf life of six months from the date of release of the

product, with a 2-4× margin of safety at first release and 2-4× margin of efficacy at expiration, was more than adequately achieved. The 2-4× margin of safety was related to absence of pulmonary side effects in pups as young as three weeks and weighing as little as 1 lb. The true expiry date of all distributed serials as measured by vaccination and challenge tests in experimental pups, extended to at least two and often three or four months beyond the predetermined dating on the label.

III. Vaccine Compatibilities and Incompatibilities

Inoculation of the vaccine is fully compatible with simultaneous vaccination against the common viral diseases of dogs. There were no significant differences in antiviral titres or in challenge protection against hookworm in dogs that received simultaneously canine hookworm vaccine and canine distemper–infectious canine hepatitis–leptospirosis canicola and icterohaemorrhagiae vaccines. There was even a suggestion that simultaneous hookworm vaccination may have had an adjuvant effect on post-vaccination virus antibody titres. Vaccination was also shown to be compatible with administration of most of the commonly used anthelmintics. There was no significant difference in protection, as measured by necropsy worm burdens of vaccinates and controls, between pups that were not treated and those that were treated with thenium-piperazine combination tablets (*Ancaris*, Burroughs-Wellcome; *Thenatol*, Jensen-Salsbery), organo-phosphorous anthelmintics (*Task*, Shell), toluene and dichlorophen (*Vermiplex*, Pitman-Moore) and tetrachlorethylene (Parke Davis). Vaccination was also shown to be successful when the dogs were simultaneously being treated with a prophylactic course of diethylcarbamazine (Squibb) or diethylcarbamazine-styryl pyridinium chloride (*Styrid-Caricide*, American Cyanamid).

The only incompatibilities observed were those which had been recorded previously from experiments in Glasgow, which were that dogs incubating and suffering from canine distemper and that were extensively affected with sarcoptic and demodectic mange, failed to respond fully or at all to vaccination.

Soon after introduction of the vaccine in the state of Florida, a need for contraindicating vaccination of older hookworm-resistant and hypersensitive dogs became apparent. While, rationally, it would seem to be unnecessary to vaccinate resistant dogs, and vaccine development was based on this logical consideration, it is not possible under field conditions to differentiate resistant from susceptible dogs in hookworm-enzootic areas. The potential therefore existed for resistant and hypersensitive dogs to be inoculated with hookworm vaccine, irrespective of need. Additionally, under practical conditions, the differentiation of hookworm disease and hookworm infection is not always made, in that evidence of hookworm infection (eggs in faeces) is regrettably often assumed to be evidence of disease. Unfortunately, soon after introduction, a number of adult dogs were inoculated with hookworm vaccine in an attempt to prevent the recurrence of low grade hookworm infection.

One of the earliest signs of hookworm infection is exhibited as the larvae

reach the lungs. In susceptible dogs exposed to an initial infection the consequences of larval migration through the lungs consists of mechanical disruption and pulmonary haemorrhage (Miller, 1971), the severity of which depends on the number of larvae in migration at any time. Normally this phase goes completely unnoticed by the owner and veterinarian. In the resistant or hypersensitive animal, the larvae appear not to escape from the alveoli in any numbers and their continued presence induces a hypersensitive reaction, rather than a haemorrhagic response. It also takes fewer larvae, especially irradiated larvae that are programmed not to complete normal migration, to cause disproportionately severe reactions in hypersensitive dogs. This was exhibited in many of the adult dogs inoculated within the first few weeks of vaccine introduction. These dogs showed respiratory embarrassment with severe coughing. This condition, however, resolved spontaneously within 3–7 days, although during this period the dogs' reactions were alarming to both owner and veterinarian. Following these events, the instructions for vaccination included a contraindication against vaccinating older dogs known to have been exposed to prolonged periods of hookworm infection and where the animal was likely to be resistant, irrespective of recurrent low level egg counts. Where hookworm transmission is year round and enzootic, for instance in the south-east United States (Florida, Georgia, coastal Texas, South Carolina, Alabama, Mississippi and Louisiana) the contraindication specified that dogs over six months of age should not be vaccinated. In northern areas, where the free-living stages of the hookworm life cycle are disrupted seasonally, the upper age limit for vaccinating was set at 12 months. The instructions, however, also stated that if the history of the dog from puppyhood was well documented in the veterinarians' records and there was little or no evidence of hookworm infection and/or hookworm disease in these records, it would probably be safe to vaccinate at any age. The adoption of this contraindication very quickly banished the problem of post-vaccination respiratory side effects.

As might also be expected, reports from some veterinarians appeared to contradict the rationale for these recommendations since issue was taken on the basis of their experiences of no side effects in adult chronic hookworm cases. Several, moreover, claimed that vaccination had prevented the recurrence of this chronic carrier problem for, at the time of reporting, six months or more. This serves to illustrate the point that in biological systems one can never be sure of being correct in all circumstances. However, since negative reports (i.e. side effects) weigh more heavily than positive attributes in establishing use patterns, the requirements for commercial success dictate that greater emphasis must be given to the former.

IV. Packaging and Distribution

It was found that the optimal temperature range for maximum shelf life of infective larvae was 10–15°C, with outside brief limits of 4–20°C. These requirements are not compatible with either refrigerator or room temperature storage. Special shipping containers were therefore developed and tested in

the laboratory against projected extremes (e.g. four days at 2–4°C three days at 35°C), and under practical shipping conditions. The shipping container comprised a cardboard box with a three-inch thick lining of urethane foam. Ethylene glycol gel bags were conditioned at 4°C, 12°C or 22°C for inclusion in the container to act as heat sink "buffers", depending on the season and geographical area of distribution. These procedures proved to be entirely satisfactory for maintenance of potency in shipped vaccine under all conditions, although logistically the system proved to be somewhat cumbersome.

Maintenance of proper storage temperatures in the veterinarian's office required invention of additional temperature control systems. Unlike the situation prevailing where parasite vaccines are used for farm animals (e.g. Dictol*) for which vaccination can be easily predicted and contracted, canine hookworm vaccine had to be immediately available to the veterinarian for a client's pup without prior contract or arrangements being made. A system was devised which utilized, with minimum expense and modifications, readily available materials. This comprised a cylindrical, insulated, plastic surfaced, one gallon container (liquid carrier) and a 25 watt electrical aquarium heater. The container was modified so as to sit easily on the shelf of a domestic refrigerator (4–8°C). The aquarium heater was adjusted to maintain 10–15°C, monitored by a simple glass-liquid thermometer. Apart from the volume of refrigerator space occupied, these units worked extremely well and maintained vaccine temperature within a range of 2·5°C for months without adjustment.

V. Practical Field Experiences

In evaluating field evidence of canine hookworm vaccine safety and efficacy, the reviewer is faced with several unique difficulties. Unlike vaccines for the control of canine distemper or rabies, canine hookworm vaccine is often administered to dogs with current or recent histories of infection. Therefore, post-vaccinal sequelae must be fully separated into two categories: (a) those related to hookworm infection; (b) bona fide vaccine-induced reactions.

Within the first category, related to pre-vaccination infection, is the chronically infected older dog. An erroneous assumption of benefit led to heavy use of canine hookworm vaccine in this class of animal during the first 3–4 months after introduction. Revised labelling appeared largely to eliminate this misuse and consequent hazard.

Unusual levels of pre-vaccination and intercurrent infection from the environment, particularly in greyhound pups that in the south are kept under ideal conditions for the hookworm life cycle and for maximum build up of infection, accounted for most of the other serious complaints. These events were usually associated with hookworm infection (positive faecals) accompanied by some signs of disease (anaemia, diarrhoea) observed before 14 days had elapsed after second vaccination. The kennel history also usually indicated that hookworm disease had been a very severe problem in

* Dictol, Lungworm Vaccine, Allen and Hanbury, London, England.

the past and that conditions contributing to this past history still prevailed during the vaccination period. The occurrence of egg counts and disease within 14 days after second vaccination indicates intercurrent infection before completion of the vaccination schedule and before immunity could be completely established by that schedule. Single vaccination (Miller, 1964) is much less effective than double vaccination (Miller, 1965b). Moreover, immunity from two vaccinations is not fully effective until 7–14 days have elapsed after completion of the schedule. Therefore, absence of protection against intercurrent infection during vaccination appeared to be a major factor in this category of complaint. There were 13 reports involving more than 71 pups that fell within this category, more than half of which were greyhound pups in only two kennels. It should be noted that even though two greyhound pups died from intercurrent hookworm challenge during the vaccination process, subsequent reports from the veterinarian indicated that, contrary to previous years' experiences in this kennel (50% or more of the pups usually died of hookworm), these were the only deaths that occurred that year.

There were a number of reports (9), involving 24 or more dogs, of a small pea-sized subcutaneous nodule after vaccination. This persisted for 7–10 days. These were observed principally in short hair breeds, did not appear to irritate the dog and they resolved spontaneously in 7–10 days. While hookworm vaccine depends for immunogenesis on live irradiated larvae, as time progresses through the shelf life of the vaccine a greater proportion of the larvae will be dead. It is believed that these small nodules were reactions to the bodies and somatic antigens of dead larvae that had not migrated from the site of inoculation. This reaction probably represented additional antigenic stimulation.

There were 12 reports involving 17 pups where hookworm egg counts were demonstrated several weeks to several months after completion of the vaccination schedule. In none of these cases were signs of hookworm disease observed (anaemia, diarrhoea, weight loss). These observations represent a major problem in education. In an earlier report (Miller, 1971), the necessity of differentiating hookworm infection from hookworm disease was emphasized. These 12 reports indicated failure to communicate adequately with every veterinarian this necessary diagnostic differentiation and the supporting background information. Hookworm disease is diagnosed on the basis of clinical signs (anaemia, diarrhoea, weight loss) with faecal egg counts being used only as confirmation and for differential diagnosis. However, it is difficult to reverse 50 years of traditional veterinary education that has established the presence of a hookworm egg as an indicator of disease.

There were three reports, involving five dogs, of delayed post-vaccination disease, that was properly diagnosed and corroborated by egg counts. The reason for these apparent "breaks" are not clear. Nevertheless, to have only five reports of this type from approximately 50000 vaccinated dogs must represent highly significant evidence to substantiate efficacy.

VI. Discontinuation

The vaccine was introduced in late 1973 in the state of Florida. By early 1974 it was also available in contiguous states of the "hookworm belt". The market area was expanded by mid-1974 over most of the Eastern half of the United States, including the south and central midwest states. By early 1975 the vaccine was available over the whole of the continental United States (except Alaska). Deliberate promotion was not, of course, conducted in areas where the free-living hookworm life cycle is usually prevented by climatic conditions, either through drought or low temperatures (desert, Northwestern and Rocky Mountain States).

Sales, however, failed to increase as much as desired. Veterinarians were reluctant to incorporate the vaccine into their routine programme immediately, preferring to vaccinate a few dogs and observe. Veterinarians also realize that most hookworm disease can be managed by anthelmintic treatment. Unrealistic expectations for successful banishing of all hookworm infection (including all disease) by vaccination, and failure to differentially diagnose infection and disease in vaccinated dogs, were responsible for many veterinarians' disappointment, however unwarranted, in the vaccine's performance. Egg counts in healthy vaccinated dogs should be viewed in the same context as post-vaccination results after use of virus vaccines with which the veterinarian is familiar (canine distemper, infectious canine hepatitis). Veterinarians do not, in routine six-monthly health examinations, attempt to isolate viruses of canine distemper or infectious canine hepatitis. They do not often see these diseases in vaccinated dogs. In any case few veterinarians are equipped to undertake virus isolations. It is, however, very easy and is, moreover, standard practice at routine check-ups to examine a faecal sample for worm eggs. Additionally many owners erroneously take their dogs to the veterinarian with a firm pre-diagnosis of "worms" on the basis of a wide variety of irrelevant signs (e.g. anal gland impaction/inflammation, any intestinal upset through overeating or improper diet, or dog "just does not look good"). Routine worming based on faecal examination, whether needed or not, also represents a major source of income to the veterinary profession. Traditionally also, worming is a primary stimulus to the owner for periodic routine visits to the veterinarian.

The increasing cost of producing this vaccine, the relatively limited shelf life (i.e. compared with inactivated bacterins and lyophilized products) and low volume of sales combined to dictate an early end to the product's marketing in 1975 after between six months and two years availability depending on area.

Acknowledgements

After spending 14 years taking this product from the embryonic stage, it is naturally disappointing to see such an end. Nonetheless the author gratefully acknowledges all the support from the University of Glasgow and from his colleagues, from Allen and Hanbury's and last, but not least, from

Jensen-Salsbery Laboratories for the opportunities of these 14 years. It has been argued that such an end might have been forecast from a knowledge of the "business" of veterinary practice. Most hookworm disease in the dog can also be controlled by anthelmintic therapy. Successful vaccines in the small animal field (e.g. virus vaccines) have become successful primarily because there is no therapeutic agent to rescue the health of the diseased animal.

References

Bolla, R. I., Weinstein, P. P. and Lou, C. (1972). *In vitro* nutritional requirements of *Nippostrongylus brasiliensis*. I. Effects of sterols, sterol derivatives and haem compounds on the free-living stages. *Comparative Biochemistry and Physiology* **43B**, 487–501.

Hansen, E. L. and Berntzen, A. K. (1969). Development of *Caenorhabditis briggsae* and *Hymenolepis nana* in interchanged media. *Journal of Parasitology* **55**, 1012–1017.

Miller, T. A. (1964). Effect of X irradiation upon the infective larvae of *Ancylostoma caninum* and the immunogenic effect in dogs of a single infection with 40 kr=irradiated larvae. *The technical report series of IAEA; Vienna* **30**, 41–47.

Miller, T. A. (1965a). Effect of route of administration of vaccine and challenge on the immunogenic efficiency of double vaccination with irradiated *Ancylostoma caninum* larvae. *Journal of Parasitology* **51**, 200–206.

Miller, T. A. (1965b). Studies on canine ancylostomiasis–double vaccination with X-irradiated *Ancylostoma caninum* larvae. *Journal of the American Veterinary Medical Association* **146**, 41–44.

Miller, T. A. (1971). Vaccination against the canine hookworm diseases. *In* "Advances in Parasitology," (B. Dawes, ed.), Vol. 9, pp. 153–183. Academic Press, London and New York.

Miller, T. A., Baker, J. D., Hein, V. D. and Stevens, F. E. (1970). A simulated field trial of an irradiated canine hookworm vaccine. *In* "Isotopes and Radiation in Parasitology II, pp. 7–13. International Atomic Energy Agency, Vienna.

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ERRATA

Lumsden et al.; Advances in Parasitology Volume 16

pp. 66-69. The following references are to be found in the journal "*Zeitschrift für Parasitenkunde*" not in the journal cited.

Janitschke *et al.* (1976)
Mehlhorn (1972a), (1972b), (1975)
Mehlhorn and Markus (1976)
Mehlhorn and Scholtyseck (1973), (1974)
Mehlhorn *et al.* (1975c), (1975e), (1977)
Rzepczyk and Scholtyseck (1976)

Reference: Heydorn *et al.* (1976) is to be found in "Berlin-Münchner tierärztliche Wochenschrift" not in the journal cited.

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