
# ADVANCES IN PARASITOLOGY

Volume 15

Ben Dawes

*Advances in*

# PARASITOLOGY

VOLUME 15

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# *Advances in*
# PARASITOLOGY

*Edited by*

BEN DAWES

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VOLUME 15

1977

ACADEMIC PRESS

London New York San Francisco

*A Subsidiary of Harcourt Brace Jovanovich, Publishers*

ACADEMIC PRESS INC. (LONDON) LTD.
24/28 Oval Road
London NW1 7DX

*United States Edition published by*
ACADEMIC PRESS INC.
111 Fifth Avenue
New York, New York 10003



Library of Congress Catalog Card Number: 62-22124
ISBN: 0-12-031715-X

PRINTED IN GREAT BRITAIN BY
ADLARD AND SON LTD, BARTHOLOMEW PRESS, DORKING

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# PREFACE

When I was approached by Academic Press and asked if I would write the Preface and a Tribute to the late Professor Dawes in this volume, I felt honoured and moved. In the Preface to the present volume I have attempted to introduce the non-specialist reader to the six full reviews and to highlight just a few of the interesting and important points. The Tribute to Professor Dawes follows the Preface.

The reviews cover a wide variety of topics. The first two papers are concerned with protozoa; one deals with the interactions between flagellates and their invertebrate hosts and the other with certain aspects of recent research on East Coast fever. The first of the four helminth reviews is concerned with the seasonal occurrence of helminths of freshwater fishes and is confined to Monogenea. The next review is also concerned with Trematoda, but here the emphasis is on the importance of the host–parasite interface. The fifth review, on filarial nematodes, deals with epidemiological and experimental studies in Brugian filariasis. The final review covers experimental aspects of the epidemiology of hydatidosis and cysticercosis.

The review by David H. Molyneux concerning the vector relationships in the Trypanosomatidae also includes monogenetic flagellates of insects as these are considered to be valuable models in enabling a better understanding of the digenetic forms.

There is an increase in the type and complexity of vectors as we proceed up the evolutionary scale with respect to host. In the freshwater and marine teleost fishes and also in elasmobranchs, the trypanosomes are transmitted by leeches and this is also the case as far as is known, in the caudate members (Urodela) of the Amphibia. However, in the Anura (frogs, toads, etc.) as well as leeches acting as vectors, sandflies are also implicated and an investigation of blood feeding arthropods could be rewarding. In the reptiles, leeches and especially sandflies act as vectors, but in addition tsetse flies act as vectors. A wide array of vectors—mites, hippoboscid flies, culicine mosquitoes and simuliids have been implicated as the arthropods involved in the transmission of avian trypanosomes. In the mammals, bugs, as well as fleas, tabanids, ticks and also keds are involved, but it is perhaps with the transmission of salivarian trypanosomes by the tsetse that most people will be familiar.

The *Leishmania*–sandfly complex is next considered and the relationship between mammalian and reptilian *Leishmania* may well prove important in understanding the epidemiology of human leishmaniasis. The review considers this wide variety of vector and parasite relationships, the theories and reasons for parasite survival and development in the various sites and their intimate association at various stages with vector tissue. The interaction of bacteria, viruses and other micro-organisms with trypanosomes in the vector is also discussed together with mixed trypanosome infections in the same host. These important aspects which may have a bearing on epidemiology have

been little studied. It is the incredible versatility and adaptability of the parasite in different regions of the host which impresses the reader. As the author points out, there has been little exploitation of the available knowledge on pathogenicity in, for example, flagellate-infected insects, or of the use of detailed vector physiology to study the interaction of parasites and host.

The review by Roger E. Purnell deals with East Coast fever of cattle which is still one of the great obstacles impeding the growth of the livestock industry in East Africa. A decade ago the UNDP/FAO East African Livestock Survey concluded that research aimed at controlling East Coast fever deserved the highest priority. As a result of this conclusion the UNDP/FAO Immunological Research on Tick-borne Cattle Diseases and Tick Control Project was set up at the laboratories of the East African Veterinary Research Organisation (EAVRO) in Kenya. The review is largely based on the work of the group at EAVRO 1967–1974, but certain aspects such as immunochemical studies involving antigenic characterisation have not been included. The group has always had the development of a vaccine as its ultimate goal, and in working towards this end have achieved a number of significant advances. Their approach has been two-fold. Firstly, they have harvested infective material from ticks and attempted to immunise cattle using this material either titrated, irradiated or in conjunction with chemoprophylaxis. Secondly they have succeeded in placing on a firm foundation the growth in tissue culture of the bovine parasitic stages and have attempted to immunise cattle using titrated or attenuated tissue culture material.

Currently, immunisation of cattle using infective material harvested from ticks in combination with chemoprophylaxis appears effective in field trials, but vaccination using tissue culture material is considered the long term objective. Further results of this work have been the improved quantitation of the parasites in tick and bovine hosts, the development of a routine serological test and a better appreciation of the complexity of theilerial species and strains which may be encountered in a field challenge.

The review on seasonal occurrence of helminths in freshwater fishes by James C. Chubb deals with the Monogenea and is the first of two parts. Monogeneans have a direct life-cycle with a single host and are found as ectoparasites on the gills, skin and external openings of fishes as well as on amphibians and turtles. A large number of observations are summarised in this review including many from the Russian literature and these are presented within taxonomic families arranged alphabetically. The seasonal studies in various parts of the world are then related to the major climatic zones and finally the presently available knowledge is summarised. Suggestions as to how further data will establish a better understanding of seasonal variations are given. The author points out that temperature may be the most relevant factor for understanding the world-wide occurrence and seasonal dynamics of the Monogenea, but current information is too scanty for detailed analysis. It is known that antibody response in fishes is dependent on ambient temperatures and this variation in response has been used to explain seasonal variations in monogenean numbers, but a good humoral response in certain species is not considered by some workers to be necessary for fluke loss. In some species

gill lesions produced by the worms contribute to worm loss. Although the effect of temperatures does not appear to offer an immunological solution to such variability, the evidence available at present suggests that as a working hypothesis the temperature of the water is of primary importance in explaining seasonal incidence.

In the intimate association between parasite and host lies the strength of the parasite and also perhaps its weakness. David A. Erasmus deals with this association in trematodes at the gut and tegument interfaces, but places emphasis on the former because of the recent comprehensive reviews dealing with the tegument. Trematodes are essentially suctorial feeders and the gut contents are egested through the mouth, there being no anus. The complex life-cycle of the trematodes, involving in some cases more than one intermediate host, necessitates alteration and adaptation of the gut in the various larval stages. These are dealt with separately before a consideration of the adult gut. Digestion in the Monogenea is intracellular in its final stages, this is in marked contrast to the Digenea and Aspidogastrea where it is extracellular and takes place in the caecal lumen. The possible antigenic nature of complex materials egested during the digestive processes in the digeneans is discussed. Although the effect of antibodies on the tegument has recently been reviewed with respect to *Schistosoma mansoni* there is little work on the possible effect of antibody on the gut in trematodes. Recent work on *Apatemon gracilis minor* has shown that there are gut changes at the time of parasite rejection and that these changes resemble immunological damage seen in the tegument of *S. mansoni*.

The evolution of the trematode gut has produced many variations and it is only with the aid of ultrastructural studies that this great diversity can be appreciated. With the advent of scanning electronmicroscopy the earlier work is being extended and attempts to correlate morphological structure with function should prove rewarding.

Brugian filariasis, a mosquito-borne disease of man in Asia, is reviewed by David A. Denham and Pat B. McGreevy. These authors point out the need for more collaboration between the experimental and applied scientist. Unlike other filarial nematodes such as *Onchocerca volvulus* and *Wuchereria bancrofti* which are only found in man, *Brugia malayi* is able to infect a variety of laboratory hosts as well. Studies in these animals have led to a better understanding of human disease. There are two basic strains of *B. malayi*—a periodic one and a subperiodic one which occurs as a zoonosis. The non-zoonotic periodic strain presents less of a problem in control. Different vectors carry these strains, but there is an area of overlap since some vectors carry both.

Diethylcarbamazine is still the only antifilarial drug available for use in man but it appears to have had little impact on world prevalence since its introduction. There is without doubt a need for a new antifilarial drug with reduced side-effects and with efficacy against the adult worm as opposed to just the microfilariae. Methods of screening compounds for activity are reviewed and improved tests described. It is suggested that the best potential candidates for new filarial drugs would be compounds with known activity against intestinal nematodes. Presently available licensed anthelmintics should

also be tested. The authors consider that the high costs for developing new drugs might be paid by an international agency since acceptable profits are unlikely to accrue to a company, even if it produces the "perfect filaricide". It is an unfortunate fact of life that the people in the developing countries with the greatest need for new filaricides are least able to pay for them. Little appears to be known about the ability of vectors to transmit low-level residual microfilariae of *B. malayi*, an important point in any control programme. There is also a need for a vaccine, but this would in the opinion of the authors, need to be able to protect against the other filarial species. With present knowledge of the disease, better control should be possible if more effort was placed on actual control and not on aspects of its distribution and prevalence.

M. A. Gemmell and P. D. Johnstone deal with the problems of hydatidosis and cysticercosis and emphasise that, as standards of meat hygiene rise, this disease complex could prevent trade expansion and development. Effective control requires a thorough knowledge of the epidemiology of these tapeworms. There are a number of difficulties in the experimental approach, but one of the most important barriers is the lack of a small animal host for the larval stages of the beef and pig tapeworms (*Taenia saginata* and *T. solium*), although the sheep metacestodes *T. hydatigena* and *T. ovis* have been found to be useful experimental models. Another problem is in defining infective patterns quantitatively, as it is difficult to find and identify the various parasitic remains in animal tissues. Factors modifying this pattern such as antibody and cell mediated immunity have been studied using *T. pisiformis* and *T. taeniaeformis* in rabbits and rats. The ability to induce immunity with viable organisms, killed or attenuated organisms, or excretory/secretory antigens is reviewed, but further work is necessary to try to link morphological larval changes with the expression of the various antigens.

Mixed tapeworm infections are also examined from the point of view of the co-existence of different tapeworm species or their use in cross immunity experiments. The potential of serodiagnosis in epidemiology has not been adequately explored. The effect on the incidence of infection in man, after the instigation of control measures is examined. This includes some interesting data on the effect of control on surgical cases of hydatid disease in man. The authors conclude that very little is known of the complex factors governing the establishment and duration of taeniid tapeworms in the definitive host.

In conclusion I would like to express my thanks to Academic Press for producing another valuable volume in rather difficult circumstances and to convey my very best wishes to the new Senior Editor Professor W. H. R. Lumsden. We can be sure that the very high standard set by Professor Dawes will be continued by Professor Lumsden and his associates.

Agricultural Research Council,
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DENYS L. HUGHES
*September*, 1976

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The late Professor Ben Dawes and his wife Jean

# TRIBUTE

# Ben Dawes 1902–1976 Emeritus Professor of Parasitology, D.Sc. (London), A.R.C.Sc., D.I.C., F.L.S., F.I.Biol.

Many will know of Professor Dawes' outstanding contribution to science, but not many will be aware of his early years and the great struggle he had before achieving the status of the scientist we came to know and respect. His example and achievements span a very wide stage of Parasitology and provide an inspiration to us all.

This tribute is divided into three sections. In the first part I will try to review his early life with its hardship and difficulties and hope that this will provide information and encouragement to students starting out today and perhaps make us all appreciate our blessings and make fuller use of our facilities and resources. In the second part I will briefly review his scientific achievements but, as many of you will be aware, this is covered in some considerable detail in the foreword by Professor Arthur to Volume 7 of *Advances in Parasitology* 1969. The final part is a comprehensive list of his publications which I was surprised to find started as long ago as 1929.

## The Man—His Youth and Background

Benjamin Dawes was born in Danehouse Terrace, Burnley on the 21st April 1902 and was one of a family of six children. He was sent to the local Abel Street Infants School when not quite three years old and reached the top class at nine years of age in the company of 12- and 13-year-olds. He would not consider attending the Grammar School, but insisted on going to work to supplement the family purse at a time when a man's wage was 30 shillings a week.

Ben went to work "half time" at 12 years of age and full time at 13 years in the weaving shed. In his notes he recalls his dread of the "knocker up" on the bedroom windows soon after 5 a.m. and the comforting wintry walks to the mill on his mother's arm and beneath her large woollen shawl as the clatter of clogs rang from the stony pavements of that Lancashire cotton mill town. He worked three to four years in the weaving shed from 6 a.m.–5.30 p.m. and at the age of 14 was working four looms. The hard life at the mill was enforced by the strict time-keeping of the weaving masters who, when the regulation half hour allocated for breakfast was up, would bolt the mill door within seconds. Many women who had gone home to get children to school and have breakfast would be locked out, and this produced hardship. On numerous occasions young Ben unbolted the door and allowed the women to slip back

to their looms. Eventually this led to Ben receiving or asking for "his cards" and he then worked on various jobs. He worked on the tramways as a cleaner, assistant storekeeper and timekeeper and tried several times unsuccessfully to get in to the Mercantile Marine. He then went back to the mill for some time but eventually went to work at Bank Hall colliery.

It was while in the sawmill at the colliery that he attended the St John Ambulance course of training and developed a liking for such subjects as human physiology and this led to his enrolment in the Technical Institute to study Zoology, Botany and Physiology together with eight other subsidiary subjects on a scholarship course. This meant intensive study four nights a week after hard physical work during the day at the sawmill. However, three years later came the reward of a Local Science Scholarship and then a National Studentship the year following which led to the Royal College of Science (Imperial College) where he commenced his studies in 1924. It was while he was a student here that the College closed for the duration of the Great Strike of 1926 and that students on limited grants found difficulties. On one occasion Ben walked most of the way home to Burnley with only an occasional lift.

## The Scientist, Scholar and Traveller

After qualifying at Imperial College, Dawes carried out research on the development of the vertebral column and the base of the skull in mammals. The mice he needed for his work were not readily available and he obtained them, through an advertisement in the *Burnley Express*, from a man who bred mice in a hut on an allotment. He was soon able to obtain foetal mice in all stages of development and this work was submitted to *Phil. Trans. R. Soc.* It was presented by Professor MacBride in 1929 and the stereograms based on graphic reconstructions from serial transverse sections are a pleasure to see. He moved from Imperial College to take up a post at Plymouth with the Marine Biological Association as Research Assistant to study the growth and maintenance of the plaice (*Pleuronectes platessa*). His first published paper originated here because earlier work presented to the Royal Society was not published until 1930. While at Plymouth he went for three months to work at the Stazione Zoologica in Naples. His work on fish still forms the basis of estimates on the food requirements of commercial fish.

In 1930 he moved to King's College at the University of London and started his long association with the college which ended on his retirement in 1969. As an Assistant Lecturer he had a very full programme of teaching and many other departmental duties which included laboratory schedules, filing, the keeping of registers, orders, and maintenance of apparatus and supervision of the Library. These hard and exacting days during the early 1930s left very little time for research, but nevertheless he obtained his D.Sc. in 1933. He became involved for at least 15 years as the Scientific Assistant in Zoology to the University of London with responsibility for all practical exams in this country and abroad. He lectured at the present Sir John Cass College as a visiting lecturer for a number of years as well as organising its first B.Sc. course in Zoology and took on examining work for Science and Medical

Scholarships. His first joint publication was with Professor J. S. Huxley (1934). During the 1939–1945 war he was evacuated to Bristol University's newly built department in which the Professor of Zoology was C. M. Yonge. It was during this period, often during air raids, that he wrote *The Trematoda* (Cambridge University Press). This book which was reprinted with corrections and additions in 1956 and 1968 is still much used as an authoritative work on the Trematoda. During these years his examining load increased as did his involvement with the developing colleges overseas in Ghana, Nigeria, Ceylon, West Indies and E. Africa. He travelled widely as a young man, but in the last 10 years or so before his retirement he travelled all over the world including two trips round the world via Malaya, Hong Kong, Japan, Hawaii and the U.S.A. He was sponsored by many bodies including the Wellcome Trust, Agricultural Research Council, C.S.I.R.O., New Zealand Department of Agriculture and the Royal Society of London. Although time for research was limited he published a number of original papers and also a Royal Society Monograph (1947). He wrote, in conjunction with G. P. Wells and others (1940), a *Practical Physiological and Physical Chemistry for Students of Zoology*. A list of his scientific publications is given at the end of this tribute but in addition he wrote a large number of semi-popular articles especially for young people. He contributed a number of chapters to *The Story of Animal Life* edited by M. Burton (1949) and also a book on *A Hundred Years of Biology* (1952). He was frequently called upon to review books on parasitology which he did in his characteristic and forthright manner. He was eminent as a teacher and a large number of former students and colleagues would testify how he filled them with enthusiasm and the desire to search. As a distinguished zoologist and parasitologist his interests were broad, but he became a specialist in the Trematoda and latterly with particular interest in the liver fluke *Fasciola hepatica*. It was in fact a common interest in this parasite which brought about my first introduction to Ben Dawes in 1958 when I went to him to discuss some experimental work on *F. hepatica* in mice and rabbits in which I was engaged. This first meeting led to a continuing friendship.

His research work was carried out with infinite and meticulous care and after much reflection, as can be seen from his papers, and needs no comment from me. Perhaps I could pick out two small facets from the many which appeal to me as giving some insight into the man. One is his characteristic way of describing in *Nature* (1952) how he observed the entire life cycle of *Diplostomum spathaceum* in a very small London pond not more than four miles from Charing Cross and the other is his review in *Nature* (1960) of Professor Hoeppli's book on real and imaginary parasites. Here Dawes states, "The book does show that many errors of judgement have gone in procession down the ages, which may have a salutary effect on parasitologists who accept over-readily what has passed before as irrefutable truth".

It was of course as editor and instigator of *Advances in Parasitology* which first appeared in 1963 that he became really widely known and recognised. He continued as editor, though in failing health up to his death. Dawes knew or came to know his contributors personally—I do not claim this

distinction, but can only say that Professor Dawes often spoke with admiration and great respect for the learning and efforts of others. I am sure he would have wanted to say a final thank you to past, present and future contributors for their endeavours and help in providing as he put it "a great fund of up-to-date information of great value in veterinary and medical science, as well as biology".

It is customary to include a photograph of the person to whom the tribute is being made, but included on this occasion is one with his wife Jean. She supported him in the great tasks he set himself and it is I am sure very fitting that the volume he was working on at the time of his death should contain it.

In *Advances in Parasitology* we see a fitting memorial to a man who gave himself unstintingly to Parasitology in its broadest and fullest sense. He was, on his own admission perhaps, a "Bluff Northcountryman" in which the nature of his well disciplined, but hard upbringing had in no way undermined his natural curiosity, resilience and determination to enjoy life. He was a man whose courage and capacity for work has left its mark.

I would like to acknowledge the help and consideration given to me by the family of the late Professor Dawes in allowing access to his files, Mrs K. Beaton our Librarian for help with the bibliography, Professor Don R. Arthur, King's College London for his kindly advice and my colleagues for useful conversations.

DENYS L. HUGHES

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# Vector Relationships in the Trypanosomatidae

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## I. Introduction

This review represents an attempt to bring together as much knowledge as possible relating to the interaction between trypanosomatid flagellates and their invertebrate hosts—the majority of those discussed are vectors. Although outside the scope of the title, in the strict sense, the monogenetic insect flagellates have been included as I feel that experimental work on these organisms can aid our understanding of the digenetic forms and will provide valuable models for later studies. Emphasis has been placed on the direct associations which occur between parasite and host rather than on indirect factors which may influence the relationship such as vector biology and food preferences. Details of the morphology of parasites in their vectors have not

been included as they can be obtained from original descriptions or standard reference texts. The biochemistry of the parasites themselves and such cyclical changes as may occur as a result of changes from vertebrate to vector have also been considered elsewhere. I hope that this review will serve as a stimulus for future work and apologise for providing yet another work on Trypanosomatidae in the wake of others that have appeared over the past decade.

## II. Lower Vertebrate Trypanosomes and their Vectors

### A. Fish trypanosomes

#### 1. *Freshwater fish trypanosomes*

The behaviour of freshwater teleost trypanosomes in leeches has been studied by few workers (Brumpt, 1905, 1906a,b; Robertson, 1912; Tanabe, 1924; Qadri, 1962; Needham, 1969). The latter work is an unpublished thesis and the present writer has deliberately included a more detailed account of Needham's (1969) findings as they represent a large amount of valuable information which might otherwise remain relatively inaccessible. Brumpt (1905, 1906a,b) studied several different species of trypanosomes in the leech *Hemiclepsis marginata*, which seems to be the most suitable vector of these trypanosomes in Europe, for other leech species which are common, e.g. *Piscicola geometra*, do not appear to be susceptible to these parasites (see Table I).

Brumpt divided the development of freshwater fish trypanosomes into two groups—those that developed in the crop (stomach) alone with no invasion of the intestine, or proboscis sheath (*T. abramidis*, *remaki*, *barbi*, *percae*, *acerinae* and *squalii*), and those that developed initially in the crop and then migrated posteriorly to the intestine where the flagellates persisted (*T granulosum*, *danilewsky*, *phoxini* and *carassii*). Before the leech was capable of transmitting the infection, the parasites migrated to the proboscis sheath. However, the validity of the species described by Brumpt should be investigated experimentally before any firm conclusions are made regarding these groupings.

Robertson (1912) studied the development of the goldfish trypanosome in *Hemiclepsis marginata*. Rapid multiplication occurred in the crop and narrow comma-shaped forms were produced. The proboscis sheath was invaded in 10 days and thenceforward the leech was infective, although immediately after an infective feed trypanosomes were not observed in the proboscis sheath. Transovarial transmission did not occur either. Robertson (1912) thought that there was an ever present residue of broad, nearly spherical trypanosomes in the leech and that there was no infective period as observed by Needham (1969); this account of the cycle of this parasite is different from that of Brumpt (1906a,b).

Qadri (1962) also observed the behaviour of *T. danilewsky* in *H. marginata*. *T. danilewsky* developed in the leech for 7 days when multiplication of epimastigotes stopped and there was transformation to long slender metacyclic trypomastigotes which were said to migrate from the crop to the proboscis

TABLE I

*Summary of available information relating to host and vectors of freshwater fish trypanosomes*

| Freshwater fish | Common name of fish | Leech host | *Trypanosoma* sp. | Sites of development: Early stages | Sites of development: Metacyclics | Authors | Remarks |
|---|---|---|---|---|---|---|---|
| *Anguilla anguilla* | eel | *Hemiclepsis marginata* | *T. granulosum* | Briefly in crop then intestine | proboscis sheath | Brumpt (1906a) Robertson (1912) | Only in crop for short time-migrates through to proboscis sheath after intestine phase |
| *Rutilus rutilus* | roach | *H. marginata* | *T. leucisci* | crop | ? | Brumpt (1906b) | Late part of cycle unknown |
| *Abramis brama* | bream | *H. marginata* | *T. abramidis* | crop | crop | Brumpt (1906b) | Infection by active migrations or regurgitation from crop as leech feeds |
| *Esox lucius* | pike | *H. marginata* | *T. remaki* | crop | crop | Brumpt (1906b) | As *T. abramidis* |
| *Barbus barbus* | barbel | *H. marginata* | *T. barbi* | crop | crop | Brumpt (1906b) | As *T. abramidis* |
| *Acerina cernua* | ruffe | *H. marginata* | *T. acerinae* | crop | crop | Brumpt (1906b) | As *T. abramidis* |
| *Phoxinus phoxinus* | minnow | *H. marginata* | *T. phoxini* | crop | proboscis sheath | Brumpt (1906b) | |
| *Cyprinus carpio* | carp | *H. marginata* | *T. danilewsky* | carp | proboscis sheath | Brumpt (1906b) Qadri (1962) | |
| *Perca fluviatilis* | perch | *H. marginata* | *T. percae* | crop | crop | Brumpt (1906b) Robertson (1912) | As *T. abramidis* |
| *Misgurnus anguillicaudatus* | loach | *Hirudo nipponica* | *T.* sp. | ? | ? | Tanabe (1924) | |
| *Tinca tinca* | tench | *Hemiclepsis marginata* | *T. tincae* | crop, proboscis sheath | proboscis sheath | Needham (1969) | |
| *Squalius cephalus* | cheuraine | *H. marginata* | *T. squalii* | crop | | Brumpt (1906b) | Described from fresh preparation only—probably as *T. abramidis* |

sheath, but did not divide further. The cycle described by Qadri resembles in some respects the observation of Needham (1969) on *T. tincae*. However, the metacyclic forms of *T. tincae* were of epimastigote configuration after the sequential division of epimastigotes following transformation of bloodstream forms.

Tanabe (1924), however, noted that in the trypanosome of the Japanese loach (*Misgurnus anguillicaudatus*) in *Hirudo nipponica*, long slender forms were produced in 3 days and division was limited to the first 3 days of the cycle. Tanabe reported that the putative metacyclics had disappeared by the 14th day, but did not record the number of divisions which preceded the production of the metacyclics.

Needham (1969) has recently studied the biology of *T. tincae* of the tench, *Tinca tinca*, in the leech vector, *Hemiclepsis marginata*. This author was able to breed clean leeches in the laboratory and study the course of infection and infectivity in the vector. In wild caught leeches *Cryptobia* infections were commonly present. These infections greatly outnumbered the trypanosomes in any one leech. Flagellates were never found at any level below the crop, which was distended after the bloodmeal. Early infections in leeches were difficult to detect as there were few parasites in the bloodmeal. Development of *T. tincae* in *H. marginata* took place initially in the crop, and the first generation of epimastigotes tended to aggregate against the crop walls. Epimastigotes divided in this site and then migrated along the crop walls, up the narrow lumen of the proboscis and passed into the proboscis sheath. Experimental leeches were kept at 15° and 20°C and development took place more rapidly at the latter temperature—flagellates were first detected in the proboscis sheath on the 5th day after infection at the higher temperature whereas at 15°C the parasites were first seen in the sheath on the 7th day. Needham (1969) found that *H. marginata* lost their infections once the bloodmeal had been digested. This disappearance of the trypanosomes from the crop and proboscis sheath coincided with the increase in numbers of bacteria and algae.

Transmission of *T. tincae* took place over a limited period of time because of this loss of trypanosomes. Uninfected tench which were fed on by leeches became infected only when fed on by leeches which had taken an infective meal 5–10 days earlier. If the leeches had been kept at 20°C instead of at 15°C, 7–14 days was the time range over which leeches could transmit the infection to tench; *Cryptobia* infections in the leeches remained, however.

The trypanosomes in the leech transformed into long thin epimastigotes in the crop and proboscis sheath, although increasing numbers of dead and degenerating parasites were seen as the infection progressed. There were only two divisions in the developmental cycle of *T. tincae* in *H. marginata* according to Needham, and these low-grade vertebrate infections gave rise to comparable low-level infections in the leech; similarly, high parasitaemias in tench produced a heavy infection in the leeches. These laboratory observations confirmed the field observations, as Needham observed very few trypanosomes in wild caught *H. marginata* and the infections that were present were greatly outnumbered by *Cryptobia*.

From the observations of these various authors certain patterns emerge relating to freshwater fish trypanosomes in leeches. It seems that at least in Europe, *H. marginata* is the most suitable host and can be infected by a variety of species of trypanosome as well as *Cryptobia*, whereas *P. geometra* has a poor vectorial capacity. *Cryptobia* infections are usually present, and as they are in a process of continuous division they swamp those species of trypanosome which have a limited number of divisions in the leech, e.g. *T. tincae* and *T. danilewsky*. Robertson's (1912) observation of an ever present residue of stem forms of the goldfish trypanosome which continually produce metacyclics, thus maintaining the infection in the leech, is not seen in other species developing in the same leech, although they have been found in marine species (Neumann, 1909; Robertson, 1907, 1910). Recently, however, Khayboulajev (1969) described cyst-like bodies resembling the forms which Deane and Milder (1966) observed in *T. conorhini*, but without specifying the trypanosome or the leech involved in this cycle.

Initial development of freshwater fish trypanosomes is confined to the crop with the infective stages in the proboscis sheath. *T. granulosum* of the eel is exceptional in that development occurs in the leech intestine. Robertson (1912) found no trypanosomes in the proboscis sheath until the blood had disappeared from the crop, whereas Needham (1969) found that proboscis sheath infections corresponded with disappearance of erythrocytes from the crop, and he felt that Robertson was referring to the disappearance of red or brown crop contents when she remarked that some flagellates took 30 to 35 days to appear in the sheath of some leeches. These observations of Robertson (1912) are contrary to those of Brumpt (1906b), Tanabe (1924), Qadri (1962) and Needham (1969); this last author found that metacyclics reached the proboscis sheath before the meal was digested, as when digestion was complete infectivity was lost, and Robertson observed this to happen after 10 days at 25°C in *T. remaki* in *H. marginata*.

Needham suggested that most fish trypanosomes may be digested, not in the crop, but in the intestine, because powerful digestive enzymes are found there (Jennings and Lande, 1967). Metacyclic forms, which are highly active, may move both forwards and backwards from the crop, but those which reach the intestine are killed whereas those which move anteriorly survive and reach the proboscis sheath eventually. Thus there may be no migratory stimulus or taxis to any particular site of development.

Jennings and Lande (1967) in their study of digestion in leeches confirmed that bacteria are present and play a part in the digestion of blood by providing enzymes which are not endogenous to the leech. The interrelationship between the bacterial flora and trypanosomes may thus be important either directly or indirectly in determining parasite behaviour and leech susceptibility.

The epithelium of the crop varies in its structure depending on the state of digestion of the bloodmeal, but there appears to be a microvillar lining similar to that in the midgut of insects. A further similarity to the insect gut structure is that the anterior region of the tube including the proboscis sheath is lined with cuticle. This is of great interest in view of the methods of attachment displayed by trypanosomes to insect cuticle. The association of fish trypano-

somes as well as *Cryptobia* in leeches must be investigated in both crop and proboscis sheath.

2. *Marine fish trypanosomes*

Khan (1974) has described in a brief note the development of the trypanosomes of the Atlantic cod *Gadus morhua*. This parasite, which was identified as *Trypanosoma murmanensis* by Khan (1972), developed in leeches of the genus *Myzobdella*. These leeches were reared at temperatures of 1°C–2°C in keeping with the waters in which the cod were living. *T. murmanensis* multiplied and reached the infective stage in *Myzobdella* in 48 days. The parasites in the leech all had yellow refractile granules in them and the configurations found were amastigotes, sphaeromastigotes, epimastigotes and metatrypanosomes. These last forms were found in the proboscis sheath.

Early work on marine fish trypanosomes was by Brumpt (1906b), Neumann (1909) and Robertson (1906, 1907, 1910). Brumpt (1906a) studied the development of *T. cotti* of *Cottus bubalis* and *T. soleae*, of the sole, in the leech *Callobdella punctata;* the parasites multiplied initially as amastigotes in the crop of the leech and later epimastigotes and metacyclics appeared in the crop, but Brumpt (1906b) never saw any evidence of invasion of the proboscis sheath by the parasites. Neumann (1909) and Robertson (1907, 1910) both studied elasmobranch trypanosomes in *Pontobdella muricata; T. raiae* was found to have an amastigote resting stage. Parasites migrated forward to the proboscis sheath where slender metacyclic trypanosome forms occurred and were believed to be infective. *T. scylli* from *Scylliorhinus stellaris* also developed in *P. muricata* (see Needham, 1969). The information available on this group of parasites is summarised in Table II.

## B. AMPHIBIAN TRYPANOSOMES

The trypanosomes of caudate amphibians (Urodeles) are, so far as is known, transmitted by leeches (Nigrelli, 1929; Barrow, 1953; Lehmann, 1952, 1958). Lehmann (1952, 1958) described the development of *T. barbari* in *Actinobdella* species and *T. ambystomae* in *Erpobdella* species. The cycles of these two species were similar in that metacyclic forms of similar structure were found in the leeches, but the site of development was not mentioned for *T. ambystomae*. *T. barbari* development took place in the crop but small numbers of metacyclic forms were found in the proboscis sheath and salivary glands (Lehmann, 1952). These two species differed in that in *T. ambystomae* a large mononucleate amastigote is produced after transformation from the bloodstream forms, which then transforms into a large epimastigote.

*T. barbari* has a similar large rounded form but in this case division occurs after an aflagellate multinucleate body has been produced. However, Lehmann (1958) does figure a multinucleate amastigote of *T. ambystomae*, which suggests that these forms may be produced in this parasite. *T. diemyctyli* (Barrow, 1953) differed from both those parasites, as the initial phase after transformation in the leech *Batractobdella picta* has an epimastigote. Barrow (1953) found also that *T. diemyctyli* developed in the crop caeca where metacyclic forms occur.

TABLE II

*Summary of available information on marine fish trypanosomes and their vectors*

| Marine fish | Common name of fish | Leech host | *Trypanosoma* sp. | Sites of development: Early stages | Sites of development: Metacyclics | Authors | Remarks |
|---|---|---|---|---|---|---|---|
| TELEOSTS | | | | | | | |
| *Solea solea* | sole | *Callobdella punctata** | *T. soleae* | crop | | Brumpt (1906b) | Never seen in proboscis sheath |
| *Cottus bulbalis* | sculpia | *C. punctata* | *T. cotti* | crop | | Brumpt (1906b) | As above |
| *Gadus morhua* | atlantic cod | *Myzobdella* sp. | *T. murmanensis* | | proboscis sheath | Khan (1974) | Development in 28 days At 1%–2%. Yellow granules in metacyclics |
| ELASMOBRANCHS | | | | | | | |
| *Scyliorhinus stellaris* | dogfish | *Pontobdella muricata* | *T. scylli* | crop | | Brumpt (1906a) | No infection seen in proboscis sheath by Brumpt |
| *Raia* sp. | ray | *P. muricata* | *T. raiae* | crop and later intestine | proboscis sheath? | Brumpt (1906a); Neumann (1909); Robertson (1907, 1910) | No infection of proboscis sheath seen by Brumpt. Early stages amastigotes. Crop stage lost when intestine invaded. Resting amastigotes present |

*Wenyon (1926) refers to this leech as *Trachelobdella punctata.*

TABLE III

*Summary of information on vectors of amphibian trypanosomes*

| Amphibian host | Vector | Trypanosome species | Sites of development | | Authors | Remarks |
|---|---|---|---|---|---|---|
| | | | Early stages | Metacyclics | | |
| ANURA | | | | | | |
| *Rana esculenta* | *Helobdella algira* | *T. inopinatum* | crop | proboscis sheath | Billet (1904) Buttner and Bourcart (1965) | |
| *Rana esculenta* | *Hemiclepsis marginata* | *T. rotatorium* | crop | proboscis sheath | Nöller (1913) | |
| *Rana* sp. | *Placobdella ceylonica* | *T. rotatorium* | crop | | Pujatti (1953) | |
| *Rana pipiens* | *Placobdella phalera* | *T. pipientis* | crop | proboscis sheath | Diamond (1965) | |
| *Rana pipiens* | *Placobdella* sp. | *T. canadensis* | crop | proboscis sheath | Woo (1969a) | |
| *Leptodactylus ocellatus* | *Placobdella braziliensis* | *T. leptodactyli* | crop | proboscis sheath | Brumpt (1914) | |
| *Bufo boreas halophilus* | *Lu. vexatrix occidentis* | *T. bufophlebotomi* | hindgut | rectum | Anderson and Ayala (1968); Ayala (1971) | Metacyclics not trypomastigote |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Bufo b. gargarizans* | *Phlebotomus squamirostis* | *T. bocagei* | hindgut | rectum | Feng and Chung (1940); Feng and Chao (1943) | No meta-trypomastigotes |
| *Rana clamitans* | *Culex territans* | *T. rotatorium* | midgut | hindgut | Desser *et al.* (1973, 1975) | Forms not infective |
| *Rana pipiens* | *Aedes aegypti* | *T. rotatorium* | midgut | | Bailey (1962) | No parasites present after 60 h |
| *Rana* sp. | *Culex quinquefasciatus* | | | | Perez-Reyez (1967) | No infective forms |
| URODELES | | | | | | |
| *Triturus torosus* | *Actinobdella* sp. | *T. barbari* | crop | salivary glands, proboscis sheath | Lehmann (1952) | |
| *Ambystoma gracile* | *Erpobdella* sp. | *T. ambystomae* | crop | | Lehmann (1958) | |
| *Triturus v. viridescens* | *Batrachobdella picta* | *T. diemyctyli* | crop | crop | Nigrelli (1929) Barrow (1953) | |

Barrow found that the high infection rate in the leech population was due to the parasitism of one leech on another and also to mother leeches taking their brooding young to a newt which acquired and transmitted the infection from the mother, as the young remained attached for 7–14 days. He failed to demonstrate transovarial transmission of *T. diemyctyli* in the leech, contrary to the findings of Brumpt (1907) for *T. inopinatum* infection of *H. algira.* Most trypanosomes of Anura are believed to be transmitted by leeches, and the information relating to this has been reviewed by Bardsley and Harmsen (1973). The developmental cycle of these parasites is initially similar, with epimastigotes being formed in the crop of the leech. Some differences in the cycles are observed in these trypanosomes, as Diamond (1965) found that two distinct types of metacyclic forms were present in the leech, elongate and stumpy. Both were found in crop and proboscis sheath. The flagellates were studied in sectioned material by Diamond, *T. pipientis* being found in crop, intestine and proboscis sheath. In crop and proboscis both attached and free forms were observed. Free individuals in the crop appeared within masses of digested blood residue. The few parasites seen in the intestine were mostly in the lumen, rarely attached.

Buttner and Bourcart (1955) reported that if leeches (*Helobdella algira*) infected with *T. inopinatum* were maintained at low temperatures, then reproduction ceased, epimastigotes persisted and metacyclic forms did not develop. Woo (1969a) observed that the developmental stages of *T. canadensis* in a species of *Placobdella* have yellow granules present. This is the only record of this phenomenon in amphibian trypanosomes, although they have been observed in *T. chrysemidis* (Woo, 1969b), a reptilian species and *T. murmanensis* (Khan, 1974). Their significance is not known.

Although amphibians are generally associated for at least part of their life-cycle with aquatic habitats and leeches play an important role in transmission of these stages, two species of Anuran trypanosome are known to develop in sandflies. Feng and Chung (1940) and Feng and Chao (1943) described the development of *T. bocagei* of the toad *Bufo bufo gargarizans* in the Old World sandfly *Phlebotomus squamirostris.* Anderson and Ayala (1968) and Ayala (1971) found that the trypanosome of *Bufo boreas* (*T. bufophlebotomi*) in California developed in *Lutzomyia vexatrix occidentis.* Both these parasites developed in the posterior region of sandflies with initial development in the midgut. *T. bufophlebotomi* produced only promastigotes.

Apart from sandflies, culicid mosquitoes have been suggested as vectors of anuran trypanosomes. However, although transformation and establishment of *T. rotatorium* from *Rana clamitans* has been observed recently in an amphibian feeding species, *Culex territans* (Desser *et al.*, 1973), Desser *et al.* (1975) have been unable to infect *R. clamitans* with these forms. These results are similar to those obtained by Bailey (1962) using *A. aegypti* and Perez-Reyez (1967) with *Culex quinquefasciatus,* who also failed to achieve transmission. The role of mosquitoes in the transmission of anuran trypanosomes must remain in doubt despite the optimism of Bardsley and Harmsen (1973). These authors recorded that in view of the general ecology of their hosts, some species of anuran trypanosomes are most likely to be transmitted by haemato-

phagous arthropods. The group of arthropods which they suggest should be examined are parasitic mites, which are found on several species of anurans which harbour trypanosomes. A correlation between the presence of an ectoparasitic mite, *Hannemania penetrans*, and trypanosome parasitaemias in three species of Anura has been observed by Brandt (1936). These ideas should be investigated.

### C. REPTILIAN TRYPANOSOMES

The earliest recorded description of the life-cycle and transmission of a reptilian trypanosome was by Robertson (1909), who described the development of *T. vittatae*, a parasite of *Emyda vittata* (soft tortoise) in a species of leech of the genus *Glossosiphonia*, the probable invertebrate host, and in the horse leech, *Paecilobdella granulosa*. In the intestine of the leech the parasites rounded up to become large syncytial bodies of four nuclei and kinetoplasts that produced epimastigotes and metacyclic trypomastigotes. The epimastigotes were found in the crop and large numbers were produced before metacyclic forms appeared.

Brumpt (1914) reported the development of a trypanosome (*T. brazili*) from a Brazilian water snake, *Helicops modestus*, in two leeches, *Placodella brasiliensis* and *P. catenigera*. Development of the forms in the leeches was confined to the stomach and no infection was observed in the proboscis sheath. Brumpt raised the possibility that the snakes became infected by eating the leeches.

The most recent description of a reptilian trypanosome in a leech was by Woo (1969b). He studied the life-cycle of a turtle trypanosome, *T. chrysemidis*. The vertebrate host was *Chrysemys picta marginata* and leeches *Placobdella parasitica* and *P. rugosa* acted as vectors. The cycle was completed in the leech in 22 days if the leeches were kept at 22°C–24°C, but if the temperature was increased to 31°C the production of metacyclic forms occurred in 14 days. The metacyclic forms were found in the crop, not in the proboscis sheath or salivary glands, and development occurred in the crop and caeca. If the leeches were deprived of a bloodmeal the infection was lost. Metacyclics would revert to epimastigotes after the second bloodmeal and divided to set up a second cycle of development. Woo believed that the leeches could therefore maintain the infection throughout if regular meals were available. Woo (1969b) did find *Cryptobia* infections in 19% of wild caught *Placobdella*, in the proboscis sheath. It is possible that the presence of *Cryptobia* may affect the development of the trypanosomes of the poikilothermic vertebrates.

Hoare (1931a,b) described the relationship of the crocodile trypanosome, *T. grayi*, to the tsetse fly, *Glossina fuscipes*. Hoare (1931a) was the first worker to recognise that the peritrophic membrane may play an important part in the development of trypanosomes in *Glossina* species. In the case of *T. grayi* the trypanosomes transformed to epimastigotes within 24 h and multiplied in this stage for 2–3 days within the endoperitrophic space of the midgut. In 4–5 days some epimastigotes had extended posteriorly within this space into the hindgut, but the flagellates had reached the open end of the tube of

TABLE IV

*Summary of information available on reptilian trypanosomes and their vectors*

| Reptilian host | Vector | Trypanosome species | Sites of development: Early stages | Sites of development: Metacyclics | Authors |
|---|---|---|---|---|---|
| CHELONIA | | | | | |
| *Emyda vittata* | *Paecilobdella granulosa* | *T. vittatae* | intestine, crop | crop | Robertson (1909) |
| *Chrysemys picta marginata* | *Placobdella parasitica* | *T. chrysemidis* | crop | crop | Woo (1969b) |
| LORICATA: CROCODYLIA | | | | | |
| *Crocodilus niloticus* | *Glossina fuscipes* | *T. grayi* | midgut, hindgut | hindgut | Hoare (1931a,b) |
| SQUAMATA: OPHIDIA | | | | | |
| *Helicops modestus* | *Placobdella braziliensis* *P. catenigera* | *T. brazili* | crop | crop | Brumpt (1914) |
| SQUAMATA: LACERTILIA: GEKKONIDAE | | | | | |
| *Hemidactylus frenatus* | *Sergentomyia babu* var. *shorttii* | *T. phlebotomi* | midgut, hindgut (Malpighian tubules) | rectum | Shortt and Swaminath (1931) |
| *Thecadactylus rapicaudus* | *Lutzomyia trinidadensis* | *T. thecadactyli* | midgut, hindgut oesophageal valve, rectal ampullae | ? | Christensen and Telford (1972) |
| *Tarentola mauretanica* | *Sergentomyia minuta* | *T. platydactyli* | midgut, oesophageal valve | midgut, oesophageal valve | Adler and Theodor (1935) |
| LACERTIDAE | | | | | |
| *Gerrhonotus multicarinatus* | *Lu. vexatrix occidentis* | *T. gerrhonoti* | midgut | anterior midgut, posterior cardia | Ayala and Mackay (1971) |
| *Sceloporus o. occidentalis* | *Lu. vexatrix occidentis* | *T. scelopori* | midgut | anterior midgut, posterior cardia | Ayala and Mackay (1971) |
| SCINCIDAE | | | | | |
| *Mabuya striata* | *Sergentomyia bedfordi* | *T. boueti* | midgut | hindgut, rectal ampullae | Ashford *et al.* (1973a) |

peritrophic membrane at its posterior extremity in the hindgut and invaded the space between the epithelium and the peritrophic membrane (the ectoperitrophic space), becoming attached in the posterior hindgut. By the 6th–8th day, the endoperitrophic space did not contain flagellates since all had migrated to the ectoperitrophic space of both midgut and hindgut. Thenceforward the parasites gradually accumulated in what Hoare (1931a,b) refers to as the ileum. It is in this region that the small metacyclic trypanosomes developed, attached to the cuticular epithelium. Hoare (1931b) found that the infection rate of experimental flies was on average about 61%, but he was of the opinion that those negative flies which he found probably did not become infected as they did not ingest any trypanosomes, which were very scanty in the blood of the crocodile. However, the initial high infection rate dropped to an average of 17·3%. Hoare suggested that the flies which were originally fully susceptible appeared to acquire a degree of immunity to the flagellate after the fifth day. Hoare (1931b) dismissed as artifacts the cysts reported by previous workers as *T. grayi*.

The first complete description of the development of a gekkonid trypanosome in a vector was by Shortt and Swaminath (1931), who described the life-cycle of *Trypanosoma phlebotomi* of *Hemidactylus frenatus* in *Phlebotomus babu* var. *shorttii*. The parasite developed massive infections of the hindgut and rectum of the sandfly in 5 days; initial multiplication took place in cyst-like bodies in the midgut.

Adler and Theodor (1935) briefly described in *Sergentomyia minuta* the development of *T. platydactyli*, a trypanosome of the Mediterranean gecko *Tarentola mauretanica*. Forty out of 43 *S. minuta* fed on infected geckos developed infections in the anterior part of the midgut which reached forward as far as the oesophagus, the gut being "choked" with parasites. *Ph. papatasi* would also develop this infection. The method of infection of geckos is probably by the flies being eaten by the lizards, but Adler and Theodor (1935) did not rule out the possibility of infection by the bite of the sandfly. During their investigations, one *Ta. mauretanica* was infected with both *T. platydactyli* and *Le. tarentolae*; sandflies fed on this gecko developed concurrent infections of both trypanosome and *Leishmania*. However, unusually for a lizard *Leishmania*, the development was in the anterior region.

Ayala and McKay (1971) described the development of the Californian lizard trypanosomes in the sandfly *Lutzomyia vexatrix occidentis*; *T. gerrhonoti* of the Southern alligator lizard *Gerrhonotus multicarinatus* and *T. scelopori* of the Western fence lizard. Both flagellates localised as long thin epimastigotes in the sandfly cardia. Occasionally, oesophageal infections were observed. Only small numbers of trypomastigotes were seen in *T. gerrhonoti*. *T. scelopori* development in the sandfly followed a similar pattern.

Ashford *et al.* (1973a) described the development of *T. boueti* of the African skink *Mabuya striata* in *Sergentomyia bedfordi*. Initial infections were of nidi of amastigotes in the midgut. Amastigotes and sphaeromastigotes of the nidi gave rise to epimastigotes. No trypomastigotes were observed. As the blood meal was voided the parasites moved to a posterior position in the hindgut and formed a palisade around the rectal ampullae. All sandflies fed on infected

skinks became infected. This development is unlike that described by Ayala and McKay (1971) for *T. gerrhonoti* and *T. scelopori*, but similar to the observations of Shortt and Swaminath (1931) on *T. phlebotomi*.

Christensen and Telford (1972) studied the development of *T. thecadactyli* of *Thecadactylus rapicaudus*, a forest gecko from Panama, in *Lutzomyia trinidadensis*. Twenty per cent of 88 wild caught *Lu. trinidadensis* were infected, mostly in the hindgut though four had midgut infections. Laboratory reared *Lu. trinidadensis* fed on infected geckos developed infection. Flagellates were found in the oesophageal valve and some in the midgut, but also in the hindgut and rectal ampullae. Epimastigotes were the predominant form although some trypomastigotes were seen. The infection of the oesophageal valve and hindgut often distended these parts of the gut, a phenomenon also observed by Shortt and Swaminath (1931) in *T. phlebotomi*.

Table IV summarises the information presently available on the trypanosomes of reptiles. Brygoo (1963) has reviewed his extensive studies on the trypanosomes of chameleons in Madagascar but no definitive information on the natural vectors of these parasites is available.

### D. AVIAN TRYPANOSOMES

A wide variety of biting arthropods have been shown to act as vectors for avian trypanosomes. Mites, hippoboscids, culicine mosquitoes and simuliids are recognised vectors to date (see Table V). However, although the behaviour of some strains is known, little information is available on the interaction between parasite and insect except in the case of *Aedes aegypti* and the *T. avium* complex in Canada studied by Bennett (1970).

Wenyon (1926) reported that mosquitoes had been suspected of being vectors of avian trypanosomes as flagellates have been seen in their salivary glands. More recently, Indian workers (Viswanathan and Bhatt, 1948; Singh *et al.*, 1950; David and Nair, 1955; Nair and David, 1956) have found flagellates which they believed were derived from birds in the salivary glands and foregut of *Culex fatigans* and *Anopheles culicifacies*. Doubt must be cast on the conclusions drawn by these workers, as their evidence for salivary gland development and transmission is scanty.

Macfie and Thomson (1929) described the development of the trypanosome of the canary in *Dermanyssus gallinae*. Within 1–2 days after the bloodmeal large epimastigotes were observed in the haemocoele, and 3–4 days later the epimastigotes had multiplied rapidly and shortened. Small metacyclic forms then appeared and filled the body cavity of the mite, the epimastigotes becoming fewer. The organs of the mite became embedded in a mass of small trypanosomes. These observations on the development of the canary trypanosome were confirmed by Manwell and Johnson (1931) and Cotton (1970). Further studies would reveal many interesting aspects of this relationship.

Baker (1956) described the life-cycle of *T. corvi* in the hippoboscid fly *Ornithomyia avicularia*. The relationship of the flagellate to the midgut is unknown, but serial sections of the hindgut indicated that the majority of forms were attached to the peritrophic membrane, which must have extended some

TABLE V

*Summary of information on avian trypanosomes and their vectors*

| Avian hosts | Vector | Trypanosome species | Sites of development | | Authors |
|---|---|---|---|---|---|
| | | | Early stages | Metacyclic | |
| *Serinus canaria* | *Dermanyssus gallinae* | "*T. macfiei*" | midgut, haemocoele | haemocoele | Macfie and Thomson (1929); Manwell and Johnson (1931); Cotton (1970) |
| European Corvidae | *Ornithomyia avicularia* | *T. corvi* | midgut | hindgut, rectum | Baker (1956) |
| Several Canadian species | *Aedes aegypti* | "*T. avium*" | midgut | hindgut | Bennett (1970) |
| Several Canadian species | *Simulium aureum*<br>*S. croxtoni*<br>*S. latipes*<br>*S. quebecense*<br>*S. rugglesi*<br>*Prosimulium decemarticulatum* | "*T. avium*" | midgut | hindgut | Bennett and Fallis (1960); Bennett (1961) |
| *Numida mitrata*<br>*Gallus domesticus* | *S. adersi*<br>"*S. impukane*"<br>*S. vorax*<br>*S. nyasalandicum* | *T. numidae* | midgut | hindgut, rectum | Fallis *et al.* (1973) |
| Anseriformes | *S. rugglesi* | *T.* sp. | midgut | hindgut, rectum | Desser *et al.* (1975) |

distance posteriorly. The parasites (pyriform epimastigotes) in the hindgut and rectum were attached by their anterior ends and Baker's photograph illustrating the attached flagellates immediately calls to mind the attachment of *T. grayi* in *G. fuscipes* (Hoare, 1931a,b) and *T. (M.) melophagium* in *Melophagus ovinus* (Hoare, 1923). The mechanism of attachment of *T. corvi* to the hindgut and rectum of *O. avicularia* is likely to be as shown by Molyneux (1975) for *T. (M.) melophagium* in these two sites.

Bennett's (1961) results on host specificity and transmission of the *T. avium* complex in Algonquin Park, Ontario, strongly suggested that ornithophilic simuliids were the natural vectors in this area. The difficulties in colonising simuliids in the area prevented further experimental studies, but *Aedes aegypti* was found to be a suitable vector in the laboratory. Bennett (1970) published the results of an extensive study of the development of the *T. avium* complex in *A. aegypti*, with particular reference to the rate of multiplication of the flagellates with respect to temperature and the relationship of the state of the bloodmeal to flagellate multiplication. Various types of trypanosomes of the *T. avium* complex were used as sources of the parasites and not all types were infective to the strain of mosquito used. Type I trypanosomes developed in *A. aegypti* Q strain; Type IA developed poorly in *A. aegypti* Q but fairly well in *A. aegypti* O; Type II trypanosomes multiplied in *A. aegypti* Q but were not infective; Type III trypanosomes did not survive or multiply in *A. aegypti* Q. These results themselves show how complex is the relationship between these parasites and their vectors, indicating that as with filaria and malaria, culicine mosquitoes may have genetic differences in susceptibility to these trypanosomes. In addition to this, not all the trypanosomes within the *T. avium* complex would develop in the same mosquito strain.

The development of the trypanosomes occurred rapidly within the bloodmeal of *A. aegypti* and metacyclic trypanosomes were found as early as 19 h after the infective feed, transformation to epimastigote having occurred 3–19 h after the feed. The initial development occurred within the bloodmeal enclosed by peritrophic membrane; only when this was broken did forms appear to colonise the hindgut and appear in the faeces. This occurred around 48 h but could be later. After the peritrophic membrane ruptured, the epimastigotes formed rosettes in the hindgut and such rosettes were found in the faeces. Bennett (1970) studied the absolute numbers of flagellates in the bloodmeal and found that in susceptible mosquitoes there was a massive multiplication within the peritrophic membrane, with each mosquito containing around 130 000 flagellates. When the peritrophic membrane ruptured, the number of flagellates present dropped to an average of 4000 per mosquito at 97 h. The flagellates became attached to the hindgut wall at this stage, particularly in the region of the caecal pouches. The flagellates did not remain in the blood nor was there a migration forward when the mosquito took another meal. However, the numbers of flagellates (which had increased to around 16 000 in the hindgut when the clean bloodmeal was being digested) dropped as the digested meal passed the hindgut. When infected mosquitoes were re-fed on an infected bird, multiplication again occurred in the midgut, indicating that the presence of flagellates in the mosquito did not inhibit multiplication in the bloodmeal.

Within the midgut during digestion of an infected bloodmeal, no parasites were seen between the peritrophic membrane and midgut wall, but they were confined to the digested part of the stomach contents. Bennett suggested, on the basis of the assessment of erythrocyte breakdown studies, that flagellate multiplication did not occur profusely until all red cells were lysed; some evidence was produced which indicated that the presence of flagellates accelerated this breakdown. The O strain of *A. aegypti* used by Bennett (1970) was more susceptible than the Q strain. In addition to this, there were more flagellates in the O strain and they persisted longer in the hindgut than in Q. Temperature studies indicated that 60°F–68°F (16°C–20°C) was optimal for flagellate development: more flagellates developed, most mosquitoes became infected, and the infection persisted for longer; however, the infection took longer to develop compared with higher temperatures. Type II trypanosomes of the *T. avium* group were different from those described above as they were unable to establish in the hindgut, only multiplying in the midgut until the peritrophic membrane ruptured. These forms were not infective to the bird. Type III parasites failed to develop at all in *A. aegypti*, though the blood form persisted up to 72 h in the midgut.

In Simuliidae the pattern of reproduction and development of *T. avium* Types II and III was similar to that observed in *A. aegypti* of Type I parasites. Recent studies on avian trypanosome vectors have been made by Fallis *et al.* (1973), who have reported transmission by *Simulium* species of *Trypanosoma numidae* of African guinea fowl, *Numida mitrata*, and chickens, *Gallus domesticus*, in Tanzania; and by Desser *et al.* (1975), who found that *S. rugglesi*, an anseriform feeding blackfly, was the vector of a duck trypanosome in Algonquin Park. The parasites developed in the posterior region and transmission was by ingestion. Table V summarises information available on avian trypanosomes and their vectors.

## III. Mammalian Trypanosomes and their Vectors

### A. Stercoraria

#### 1. Subgenus *Herpetosoma*

(a) *T. rangeli*. *Trypanosoma* (*Herpetosoma*) *rangeli* is one of the most interesting of mammalian trypanosomes. It is a parasite of man and various mammals in Central and South America and is transmitted by the bite of reduviids, usually *Rhodnius prolixus*. The biology of this parasite has been reviewed recently by Hoare (1968, 1972) and D'Alessandro (1976). The organism was first discovered by Tejera (1920) in *Rhodnius prolixus*. The transmission is by the bite of *R. prolixus* after development in the salivary glands; infection of the salivary glands occurs after haemocoele invasion.

*Rhodnius prolixus* is the most common and susceptible bug to infection with *T.* (*H.*) *rangeli*, but recently other vectors have been identified in different areas under natural conditions: *Triatoma dimidiata capitata* in Colombia (Marinkelle, 1968b); *R. pallescens* in Panama (Zeledon, 1965); *R. brethesi*, *Eratyrus mucronatus*, *R. pallescens* and *T. d. capitata* in Colombia

(D'Alessandro *et al.*, 1971); *R. ecuadoriensis* in Peru (Herrer, 1964); and *Panstrongylus megistus* in Brazil (De Lucena and Vergetti, 1973). D'Alessandro (1972) recently found that *R. neglectus*, *T. protracta*, *T. patagonica* and possibly *T. infestans* were suitable experimental vectors in Colombia.

These studies indicate that *T.* (*H.*) *rangeli* is less restricted in the invertebrate host than was previously believed. In addition we should also remember that *T.* (*H.*) *rangeli* itself is probably a species complex with a wide distribution both in mammals and bugs. This group of organisms may include many of the South American primate parasites. Zeledon (1970), for example, considers that *T. myrmecophagae* and *T. diasi* are synonyms of *T.* (*H.*) *rangeli*.

*T.* (*H.*) *rangeli* was first shown to be pathogenic to its vector, *R. prolixus*, by Grewal (1957), who also found the trypanosome to be pathogenic to the bed-bug *Cimex lectularius*. Grewal found that first instar nymphs had a higher mortality than other instars and that bugs with a haemolymph infection were unable to moult. The unnatural host *Cimex lectularius* was found to be more susceptible to *T.* (*H.*) *rangeli* than the natural vector. The degree of infection of the haemolymph was proportional to the mortality. Grewal concluded that mortality was not connected with gut-wall penetration, a belief substantiated by Watkins' work (1971a, b) (see later). Grewal indicated that future studies should concentrate on the lowering of amino acid levels in the haemolymph and the effect this has on the moulting process. Ormerod (1967a) did confirm that changes in amino acids occurred in infected bugs. Two different isolates of *T.* (*H.*) *rangeli* were used by Ormerod and haemolymph amino acid levels were analysed. A lethal Venezuelan isolate which initiated heavy haemolymph infections produced a general fall in amino acid levels but a ten-fold rise in the concentration of glycine, alanine and isoleucine, a hundred-fold rise in taurine and a five-hundred-fold rise in aspartic acid. Ormerod suggested that these increases were due to metabolic malfunction; he regarded as significant the gradual increase in amino acid levels caused by the non-pathogenic Colombian strains and believed that the difference in response was due to the "immune" response of the bug to the more virulent strain. It is possible that some triatomids have a natural immunity against *T.* (*H.*) *rangeli* in the haemocoele; Zeledon and de Monge (1966) found that if Indian ink was inoculated into the haemocoele of *Triatoma infestans* in experimental infections and haemocyte phagocytosis was blocked, heavier infections resulted, whereas in normal bugs the infection was light and transient.

Grewal's findings (1957) of pathogenicity in *T.* (*H.*) *rangeli* infected bugs was confirmed by Tobie (1961) in laboratory work and by Marinkelle (1968a) in the field. The latter author concluded that *T.* (*H.*) *rangeli* represented a most important mechanism of natural control of *R. prolixus* in certain areas. This clearly is of importance in the epidemiology of Chagas' disease. The interaction of mixed infections in nature has been discussed by D'Alessandro and Mandel (1969). The presence of *T.* (*S.*) *cruzi* in the intestine did not prevent *T.* (*H.*) *rangeli* from invading the haemolymph and salivary glands. But these latter authors found that bugs infected with both parasites took a bloodmeal significantly less frequently than those without infections. The life-cycle in the bug has been intensively studied (D'Alessandro, 1963; Tobie, 1965, 1968) and

is still somewhat controversial. It is now believed (Hoare, 1972) that epimastigotes are produced in the midgut of the bug, penetration of the midgut epithelial cells occurs (Rey-Matiz, 1941) with resultant haemocoele infections, and from here epimastigotes invade salivary glands where metacyclics are produced. The hindgut forms found in some infected bugs are thought to be the result of the bug swallowing metatrypanosomes from the salivary glands (Zeledon, 1970). The forms found in the hindgut are believed not to be infective to mammals. Furthermore, *T.* (*H.*) *rangeli* does not colonise the insect rectum as *T.* (*S.*) *cruzi* does.

The detailed physiological changes which occur in *Rhodnius* infected with *T.* (*H.*) *rangeli* have been studied by Watkins (1971a,b). In the first paper (Watkins, 1971a) she described the physiological effects of the infection on the bug and showed that, at least in the strain which she used, the trypanosomes invaded many tissues of the body. The original papers should be consulted by interested workers, as Watkins introduces the concept of effects of a parasite resulting in complex interactions which result in the death of the bug from a variety of causes. This type of study has been greatly aided by the great volume of knowledge about the physiology of *R. prolixus* (see Wigglesworth, 1965).

Watkins (1971a), in contrast with earlier workers, found that the Venezuelan strain ($XX^{5}4$ obtained from Dr E. J. Tobie) of *T.* (*H.*) *rangeli* produced infection within many of the cell types which were bathed by the haemocoelic fluid; particularly favoured sites were gut musculature, fat body, epidermis and salivary glands. Tracheal and nerve cells became hypertrophied and severely damaged as a result of the infection with *T.* (*H.*) *rangeli*. Evidence was also found indicating that the symbiote *Nocardia rhodnii* was inhibited by the presence of the trypanosome. *N. rhodnii* is known to provide essential vitamins to the bug (Lake and Friend, 1968; Auden, 1974). Thus the resultant vitamin deficiency will inevitably set up a series of changes resulting in moulting deformities, for the deformities observed in symbiote-free bugs were similar to those observed by Watkins in *T.* (*H.*) *rangeli* infected bugs (Geigy *et al.*, 1953; Harrington, 1960). The moulting processes of *R. prolixus* are also likely to be disturbed, with a resulting increase in intermoult periods, by the presence of intracellular *T.* (*H.*) *rangeli* in the haemocytes, for these cells are essential for the synthesis of moulting hormone from the thoracic glands (Wigglesworth, 1965). Watkins (1971a) believes that flagellates clustered around the lamellae of organs may possess an enzyme that enables them to penetrate this layer and then enter cells. When entering the haemocoele the changes in permeability associated with their passage through the basal lamina will result in an increase in haemocoele volume.

Watkins (1971b) also studied the effect of *T.* (*H.*) *rangeli* on the excretory functions of *Rhodnius prolixus*. There was a marked decrease in excretion compared with control uninfected bugs. The decrease was particularly apparent in bugs with haemocoelic infections. The factors which brought about this inhibition of excretion were suggested as tissue damage, lack of diuretic hormone or the presence of a chemical inhibitor in the haemocoele, changes in osmotic pressure, or a combination of these. The tissue damage which results

from the presence of *T. (H.) rangeli* in *R. prolixus* was listed by Watkins (1971b) as (1) necrotic and distintegrating gut muscles, (2) blistering of neural lamellae with localised loss of nerve substance, (3) heavy infection of tracheal cells, (4) presence of many parasites in mesometathoracic ganglion, (5) localised necrosis of Malpighian tubule cells, (6) destruction of brush border of the Malpighian tubule, (7) destruction of basal lamella of Malpighian tubules, (8) closure of lumen of these tubules. Clearly, if all these effects are summated, spectacular changes will occur in the physiological processes. The damage to nervous tissue of some organs responsible for the initiation of hormone release, or damage to the neurosecretory cells of the ganglion, may result in reduction of circulatory diuretic hormone (Maddrell, 1964).

However, experimental implantation studies indicated that the inhibition of excretion was directly due to the presence of a chemical inhibitor in the parasites themselves. These classical studies of *T. (H.) rangeli* in *Rhodnius prolixus* utilising our knowledge of *Rhodnius* physiology point the direction in which further studies on insect–trypanosome interactions should be turned. Whether or not all the changes described by Watkins (1971a,b) occur in naturally infected *R. prolixus* is another problem, and wide strain variation in effects is known (Tobie, 1961; Ormerod, 1967a). However, if only some of the described changes occur it would be sufficient to initiate a series of pathological processes which might end in death. Not only is it likely that strains of the trypanosomes vary in pathogenicity but also that different bug strains will vary in their response to a given trypanosome strain.

Nothing is yet known of the fine structure of the relationship between *T. (H.) rangeli* and the various sites it affects in *R. prolixus*. It would be particularly interesting to examine *T. (H.) rangeli* in the salivary glands of bugs to see whether changes occurred in preadaptation of metacyclic trypanosomes to life in the vertebrate and the attachment mechanisms employed by the parasites to the salivary gland epithelium.

(b) Other species of *Herpetosoma*. Molyneux (1976) has recently reviewed the biology of the trypanosomes of the subgenus *Herpetosoma* and described the morphology of these parasites in their vectors, which are usually Siphonaptera. He also tabulated the vectors of those species which have been studied and the sites of development within the fleas. Between the writing of this review and the current article, no new work has been completed, and other details of vectors and life-cycles of this subgenus are given by Hoare (1972). However, in relation to the specific problem of host–parasite relationships and possible interactions, several points of interest will be mentioned in this section in order to place this subgenus within the context of the relation between the other subgenera and their vectors. The morphogenetic pattern of transformation is similar in all those rodent trypanosomes which have cycles of development in fleas (see Hoare, 1972; Molyneux, 1976). However, it does appear that establishment of epimastigotes can take place in either hindgut and/or rectum. Molyneux (1970) pointed out that in trypanosomes of the rodent subfamily Murinae (*T. lewisi*, *T. musculi*), development occurred in the rectum as well as occasionally in the hindgut, but in the Microtinae,

establishment and development in fleas was in the hindgut with no colonisation of the rectum. Although this cannot be regarded as an absolute rule the pattern fits well with the observed life-cycles and can be related to other aspects of the biology in the mammal host.

The problem of the intracellular forms of *T.* (*H.*) *lewisi* in flea midgut cells was discussed by Molyneux (1969b, 1976). The species of *Herpetosoma* in which such forms have not been found and the species of flea examined were listed by Molyneux (1969b). Minchin and Thomson (1915) first reported these forms in *Nosopsyllus fasciatus* infected 24 h–72 h previously with *T. lewisi.* The problem was later studied by Molyneux (1969b), who found that two strains of *T.* (*H.*) *lewisi* produced these forms very infrequently in fleas whereas two other strains he studied did not. Garnham (1955), using the tropical rat flea *Xenopsylla cheopis*, did not find intracellular forms, but he did find that at the time the forms were found most frequently by Minchin and Thomson (1915) (around 48 h after the infective feed) there was a significantly higher mortality in the fleas fed on an infected rat compared with those in the control group fed on an uninfected rat. Garnham (1955) carried out this experiment on the basis of the comment by Wenyon (1926) that the infected epithelial cell was reduced to a mere membrane and pathogenicity was suspected. The higher mortality observed by Garnham occurred during the first 72 h, twenty fleas dying out of 62 which fed on the infected rat. During the subsequent week death rates in the two batches of fleas were approximately the same. A second experiment confirmed the earlier observations; in this case, fleas were left in contact with the two rats for only 24 h, and again a higher death rate was observed on the first day after the feeds in the fleas fed on *T.* (*H.*) *lewisi* infected rats compared with fleas fed on uninfected control rats. However, a second feed of both batches on infected and uninfected rats showed that there was no subsequent differential mortality in the two groups. The significance of the intracellular forms of *T.* (*H.*) *lewisi* in fleas has been discussed by Molyneux (1969b, 1976). It was suggested that the parasites invaded midgut cells as part of a process which may result in invasion of the haemocoele and subsequently more efficient salivary gland transmission, as in *T.* (*H.*) *rangeli.* The pathogenicity observed by Garnham (1955) in *T.* (*H.*) *lewisi* implies that the process may be of recent origin for a parasite which appears to be well adapted at least to its mammalian host. An alternative suggestion which has been made in the light of recent observations of forms of cyst-like bodies of *T. conorhini* in culture (Deane and Milder, 1966) and similar forms in *T.* (*S.*) *cruzi* infected bugs (Brener, 1972), is that the intracellular forms of *T. lewisi* may be involved, as these authors suggest, in genetic exchange between organisms as kinetoplasts and nuclei from several organisms may be in contact without separation by parasite cell membranes.

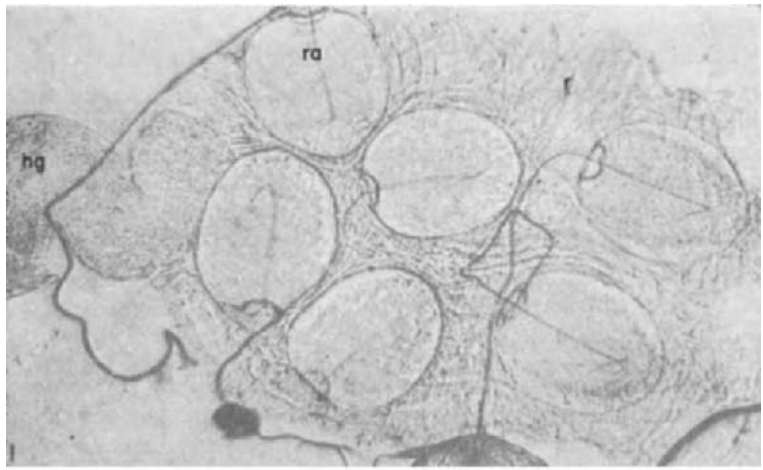
A further interesting result was reported in an unpublished thesis by Irving (1965). This author investigated the infection rate of fleas, *Xenopsylla cheopis*, fed on rats infected with *T.* (*H.*) *lewisi* at different stages of the infection and reported that it was related to the age of the infection in the rat rather than to the numbers of parasites taken in by the flea. Irving (1965) found that if fleas were fed on rats infected with adult parasites in the blood, infection rates in

the fleas *X. cheopis* were between 50%–80%. Infection rates were lower if fleas fed on reproductive stage infections in the rat (Irving, 1965). A similar infection rate was observed by the author in *N. fasciatus* fed on rats with adult infections of *T.* (*H.*) *lewisi*. Deane (1969) suggested that the monomorphic adult forms of *T.* (*H.*) *lewisi* are those "competent to infect the invertebrate".

Molyneux (1969a) investigated the method of attachment of *T. lewisi* to the rectum of the flea *N. fasciatus*. The rectum, which contains six rectal glands or ampullae, is a highly active folded bulb-like organ (Fig. 1). Sections revealed that the flagella of free-swimming nectomonads were interdigitated between the folds of the rectum and parasites also were found wedged in these folds (Fig. 2). Haptomonad epimastigotes were attached by what Molyneux (1969a) called at this time a zonula adherens but which has now been called a hemidesmosome (Brooker, 1971a; Vickerman, 1973; Killick-Kendrick *et al.*, 1974), a term which has been adopted to describe this type of junction between flagellar membrane and cuticle. The flagella of these forms had an expanded intraflagellar region, another feature which has since been reported consistently in flagellates *in situ* in insects. Rosettes of parasites in the rectum were found as a result of incomplete division; several axonemes were found within single flagellar sheaths, and it was suggested that these maintained the integrity of rosettes which then maintained their position in the rectum more easily, so a continuous supply of metacyclics could be produced from a constant reservoir of epimastigotes (for it is thought that once a flea is infected with *Herpetosoma* trypanosomes it remains infective for life). Some *Herpetosoma* species are found established in the hindgut triangle and hindgut of fleas and not in the rectum. In a small proportion of *T.* (*H.*) *lewisi* infected fleas, parasites are found in this site. The method of attachment in this region is not known, but Molyneux and Ashford (1975) have observed that an unknown trypanosomatid in a rodent flea *Peromyscopsylla silvatica spectabilis* is attached by a desmosome-like attachment. The flagellum of this parasite was greatly expanded and occupied folds of the epithelium. Although Molyneux and Ashford (1975) believed that this parasite was not a trypanosome, the method of attachment employed in this site (flea hindgut) by *Herpetosoma* parasites is likely to be similar.

Although the majority of known species of this subgenus are transmitted by fleas, Frezil and Adam (1971) and Ewers (1974) have implicated other possible vector groups. Frezil and Adam (1971) believe that *T.* (*H.*) *perodictici* of *Galago demidovi* in Congo-Brazzaville may be transmitted by *Anopheles cinctus*. The cycle of development terminated in the posterior region but differed from rodent species of *Herpetosoma* in Siphonaptera in that the epimastigotes reached 50 $\mu$m in length. Ewers (1974) described a new trypanosome, *T.* (*H.*) *aunawa*, from an insectivorous bat, *Miniopterus tristis*. This organism is believed to be transmitted by a terrestrial leech, *Philaemon jawarensis*. Flagellates in the leech contained "hemozoin-like" granules. This finding is interesting, as other authors have found similar inclusions in leech transmitted trypanosomes (Woo, 1969b; Khan, 1974).

Although the observations of possible mosquito and leech transmission of



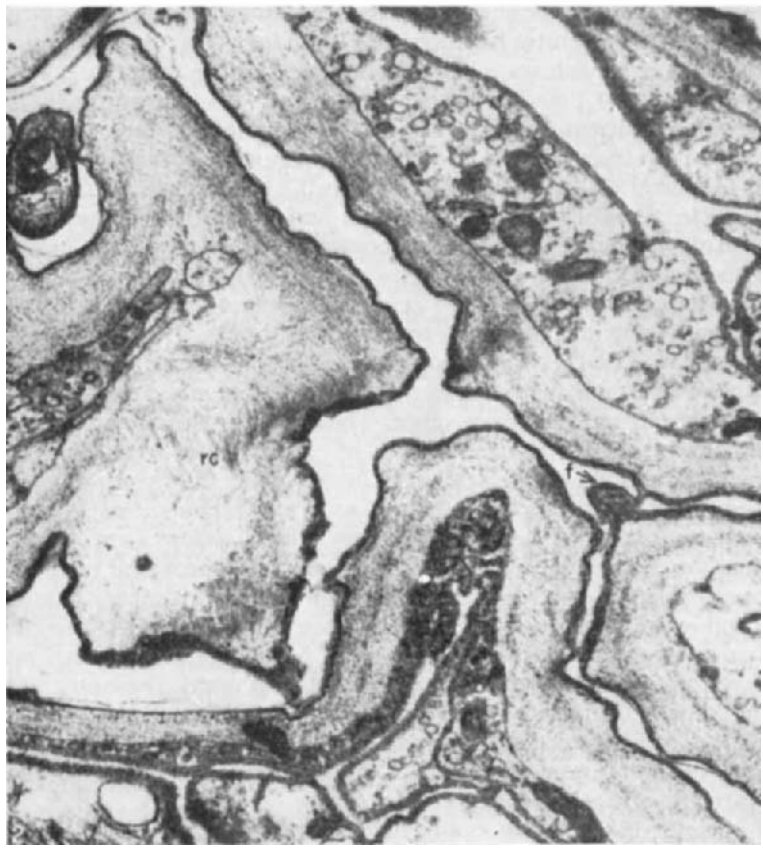




FIG. 1. Rectum (**r**) of the flea *Nosopsyllus fasciatus*. Rectal ampullae (**ra**); hindgut (**hg**). Note the thin lines on the surface of the rectum indicating the highly folded nature of the wall of the structure. × 300.

FIG. 2. Rectum of *N. fasciatus* infected with *T. lewisi*, showing highly folded nature of rectal wall which is lined with cuticle (**rc**). Note *T. lewisi* (**p**) (top left-hand corner) wedged in fold of wall and a flagellum (**f**, arrowed) of a nectomonad inserted between folds. × 20 000,

*Herpetosoma* trypanosomes were made in primates and Chiroptera respectively, it should be noted that these mammalian orders are not common hosts of this subgenus, where the usual development cycle involves rodents and Siphonaptera. It seems that in the two examples quoted above, as well as in *T.* (*H.*) *rangeli*, the basic rodent trypanosome stock has diversified to other hosts where Siphonaptera are not found to be suitable vectors and alternative ones may be required.

## 2. Subgenus *Megatrypanum*

The subgenus *Megatrypanum* comprises those species of mammalian trypanosomes which are typically large, of variable size but possibly up to 120 $\mu$m in length, with a kinetoplast relatively close to the nucleus and a pointed posterior end. Trypanosomes of this subgenus are not normally associated with pathogenic conditions. Reviews of the biology of this group of trypanosomes are given by Keymer (1969), Wells (1972a) and Hoare (1972).

There has been much speculation about the possible vectors of the cattle trypanosome, *T.* (*M.*) *theileri*. Hoare (1972) concluded that no single worker had provided sufficient evidence to incriminate any one group of arthropods as vectors of this trypanosome. On the basis of the work of Nöller (1925, 1931) and Kraneveld (1931) particularly, it is generally believed that *T.* (*M.*) *theileri* is transmitted by Tabanids (*Haematopota* and *Tabanus*) and that infection is the result of contamination of mucous membranes by small metacyclic forms which developed in the hindgut.

A recent publication (Burgdorfer *et al.*, 1973) provides evidence that *T.* (*M.*) *theileri* can develop in, and may be transmitted by, ticks. These authors found that 19 out of 258 (7·4%) *Rhipicephalus pulchellus* had haemolymph infections of a trypanosome, and one out of 69 (1·4%) of *Boophilus decoloratus*. The ticks were collected from Ethiopian cattle. The organism was identified on its morphology as *T.* (*M.*) *theileri*. Masses of epimastigotes were found in the haemolymph and all configurations characteristic of *Trypanosoma* were observed. In two *R. pulchellus*, amastigotes and sphaeromastigotes were abundant and associated with plasmatocytes. All tick organs were invaded but particularly ovarian tissues, though no evidence of transovarial transmission was found. Salivary glands were moderately infected with sphaeromastigotes transforming to trypomastigotes which may represent metacyclic forms. Invasion of connective tissues, epithelium of Malpighian tubules and muscle cells by epimastigotes and sphaeromastigotes also occurred. These observations confirm the view expressed by Wenyon (1926) that O'Farrell (1913) observed *T.* (*M.*) *theileri* in *Hyalomma aegyptium* and not a *Crithidia* as he reported. Carpano (1932), however, observed *T.* (*M.*) *theileri* in a species of *Hyalomma*, as did Arifdzhanov and Nikitina (1961).

These results indicate that both tabanids and ticks can transmit *T.* (*M.*) *theileri*. Tabanids have a cycle in the gut with posterior region development and contaminative transmission, and ticks have a generalised haemolymph and organ infection, the parasites invading the salivary glands and transmission perhaps occurring by bite. Recent studies supporting tabanid trans-

mission of *Megatrypanum* species have been obtained by Davies and Clark (1974) and Krinsky and Pechuman (1975).

*T.* (*M.*) *melophagium*, a parasite of sheep, which is transmitted by the sheep ked *Melophagus ovinus*, was thoroughly studied in the vector by Hoare (1923). In this study the development of the parasite took place initially in the midgut where epimastigotes are attached to the brush border of the midgut cells, as well as in rosettes in the lumen of the gut. The epimastigotes in the midgut are elongate with a long free flagellum. However, in the iliac bulb and the rectum, pyriform epimastigotes as well as metacyclic forms are attached to the cuticular lining of the epithelium. The flagellum of these forms appears not to be free. The details of the association of *T.* (*M.*) *melophagium* with various parts of the gut of *M. ovinus* have been studied by Molyneux (1975). In the midgut of the ked, flagella and the anterior ends of the epimastigotes are inserted between the microvilli. No hemidesmosome attachment zones were observed between flagella and microvilli and the flagella did not possess expanded intraflagellar spaces. Mycoplasma-like organisms which were initially described in *Melophagus ovinus* and named *Rickettsioides melophagi* (Kreig, 1961) were also present amongst these microvilli and flagella, and it is possible that these organisms play a role in anchoring parasites in this site. In the iliac bulb (hindgut triangle) of the hindgut, masses of epimastigotes and metacyclic parasites are attached to the cuticle by hemidesmosomes. Very little of the cuticular lining of the gut of the vector in this region is not covered with flagellates. All possess expanded intraflagellar areas, and dense hemidesmosomes are associated with the inner surface of the flagellar membrane in contact with the cuticle. The axonemes terminate close to the hemidesmosomes with their axis at 90 degrees to the epithelium. Hemidesmosomes of a similar nature to those found in the iliac bulb were found in parasites in the rectum of *M. ovinus* despite differences in cuticle structure lining these organs. The various mechanisms of attachment are illustrated in Figs 3–6.

The vast numbers of *T.* (*M.*) *melophagium* found in *M. ovinus* were claimed by Nelson (1956) to be a cause of pathogenicity to the sheep ked. Nelson found that large numbers of keds died as a result of blockage of the posterior midgut by numerous parasites. The symptoms observed were a swelling of the abdomen, the development of a reddish colour over the entire insect due to the escape of sheep erythrocytes into the haemolymph, and a change of colour of the iliac bulb from brown to white. This white colour is generally observed in insect guts when masses of flagellates are seen. Nelson reported that the anterior midgut had undigested blood present but the posterior midgut was emptied of digestive contents and blocked with parasites. Excretory products were present in the iliac bulb and the Malpighian tubules were greatly distended but did not contain parasites. Nelson (1956) concluded that *T.* (*M.*) *melophagium* is pathogenic to *M. ovinus*, but Hoare (1972) does not agree with this interpretation on the basis of his experience of this trypanosome and the lack of definitive evidence from Nelson (1956). There may well be, however, geographical variation in behaviour of strains of trypanosome or vector, provoking different host–parasite relationships. *T.* (*M.*) *melophagium* does provide an excellent model system for the study of trypanosomes in insects.

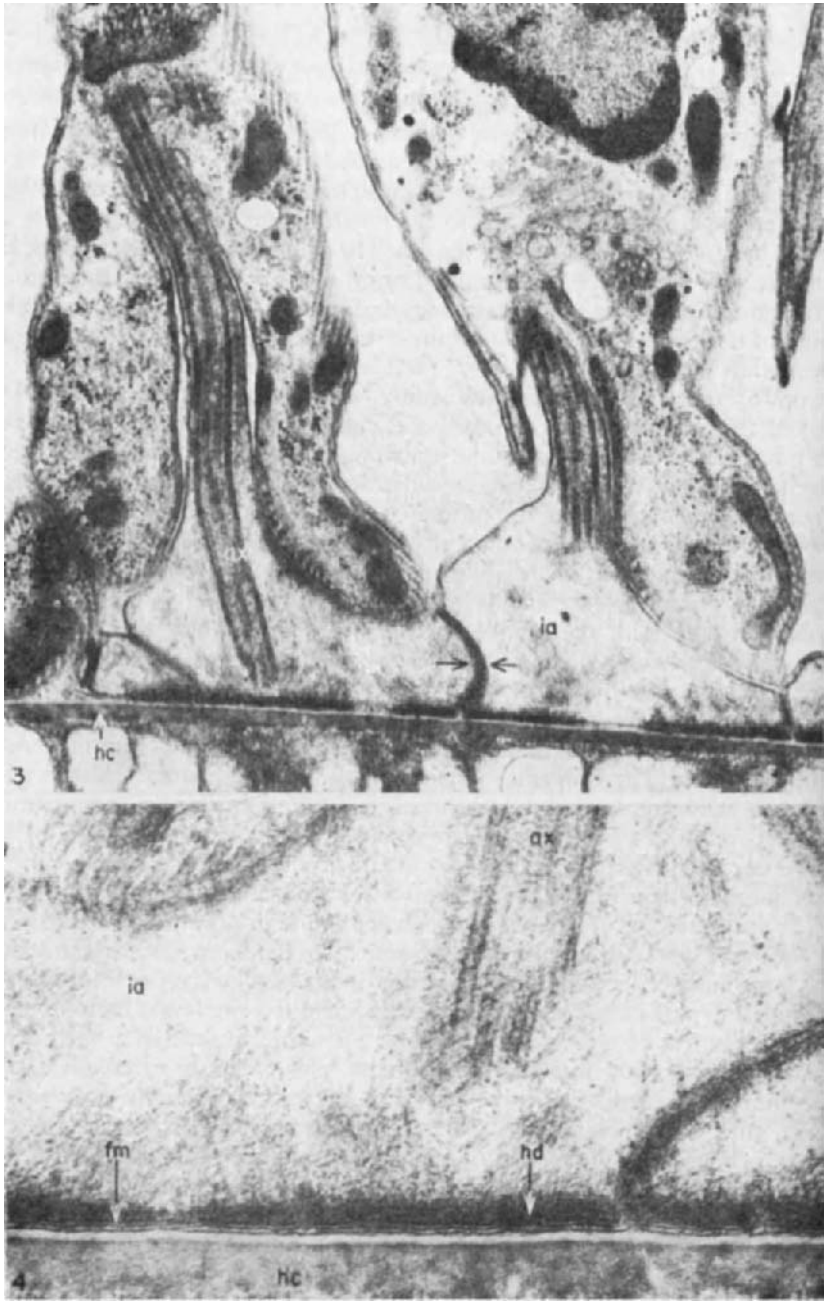



FIG. 3. *T.* (*M.*) *melophagium* epimastigotes attached to the hindgut of *Melophagus ovinus*, the sheep ked. Note expanded intraflagellar area (**ia**), and flagellar membranes of the parasites attached to each other (laterally between arrows). Hindgut cuticle (**hc**, arrowed), axoneme (**ax**). × 22 500.

FIG. 4. *T.* (*M.*) *melophagium* in *M. ovinus* showing detail of hemidesmosome (**hd**, arrowed), between flagellar membrane (**fm**, arrowed) and hindgut cuticle (**hc**). Intraflagellar area (**ia**), axoneme (**ax**). × 100 000.

(Figures 3 and 4 from Molyneux, 1975; reproduced by kind permission of the Editors of *Acta trop.*)

Williams (1976) has studied *Trypanosoma* (*Megatrypanum*) *leonidasdeanei* in *Lutzomyia beltrani*, a cave-dwelling sandfly from Belize. Massive posterior hindgut infections were found in flies and extended into the rectum and surrounded the rectal ampullae. Some infections were also found in the midgut and cardia. The posterior infections appeared to block the gut lumen by forming a stationary plug of flagellates. Williams found that those flies infected with flagellates suffered a disruption of gonotrophic concordance, possibly due to the competition for available metabolites between ovary and parasites. No mortality was associated with massive infections of this trypanosome in *Lu. beltrani*.

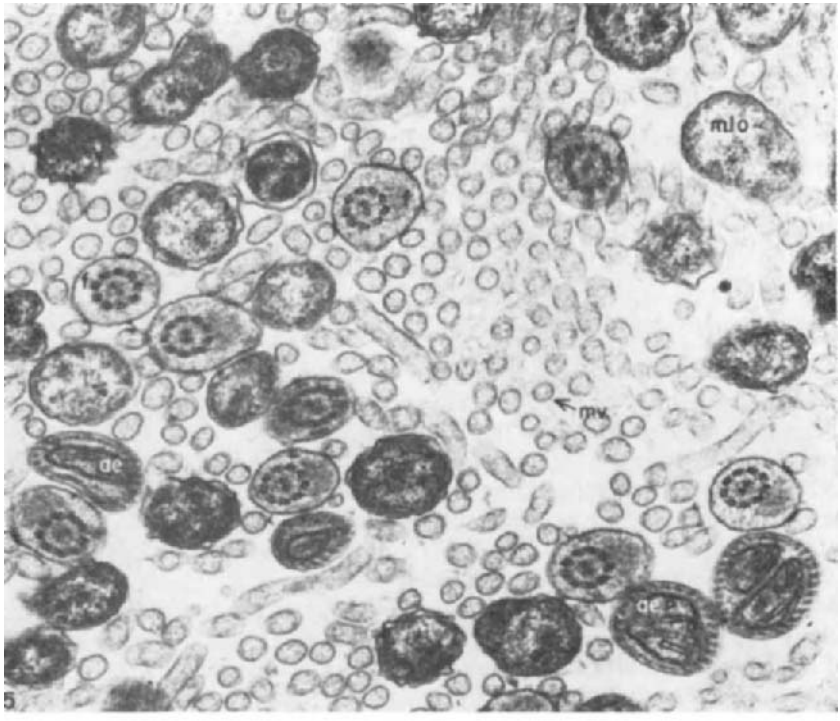
3. Subgenus *Schizotrypanum*

All aspects of the biology of *T.* (*S.*) *cruzi* in the various vector species of reduviid have been reviewed by Hoare (1972) and Brener (1973). It is not therefore proposed to discuss this subgenus in detail, as it has been dealt with specifically by Brener in other papers in addition to the above review (Brener, 1971, 1972). Aspects of the biology of *T.* (*S.*) *cruzi* in the vector have been or will be discussed in various sections of this review. This pertains to concurrent infections of *T.* (*S.*) *cruzi* and *T.* (*H.*) *rangeli* (D'Alessandro and Mandel, 1969; see Section IIIA1a); *Nocardii rhodnii* and *T.* (*S.*) *cruzi* in bugs (Mühlpfordt, 1959), the bacterial flora of bugs (Cavanaugh and Marsden, 1969), *Blastocrithidia triatomae* (Cerisola *et al.*, 1971), and a *Candida* infection of *T.* (*S.*) *cruzi* cultures (Lehmann, 1970) (all in Section VI).

Although the ultrastructure of *T.* (*S.*) *cruzi* has been studied in bugs by Sanabria (1966) and Brack (1968), details of any association between the gut wall and the parasites were not contained in these reports.

Studies of particular interest which should be followed up on the cycle of *T.* (*S.*) *cruzi* in the vector concern the possible genetic factors influencing susceptibility to infection. Phillips and Bertram (1967) observed differences in susceptibility to infection in individual bugs. Some bugs remained negative after feeding on 4 or 5 infecting meals and some could only be infected after repeated feedings on rats with heavy parasitaemias. Selective breeding of the progeny of those bugs which failed to become infected after taking infected blood resulted in a generation of bugs which had a significantly lower infection rate (57%) compared with the control vector population (80·5%). Clearly, this work should be followed up.

The work of Brener (1972) has suggested that the initial development of *T.* (*S.*) *cruzi* in the stomach of *Triatoma infestans* can be interpreted as potentially enabling genetic recombination to occur. Brener observed rounded or pear-shaped organisms attached in pairs (as did Lapierre, 1972b), some of which appeared to show cytoplasmic bridges and membrane leaks. Later fusion of the parasites and disorganisation of nucleus and kinetoplast occurred, followed by thickening of the borders of the parasite pairs and thus parasite detachment. Brener (1972, 1973) speculated that this process may represent a method of reproduction involving genetic exchange. Previous work on this subject by Amrein (1965) using drug resistant strains failed to demonstrate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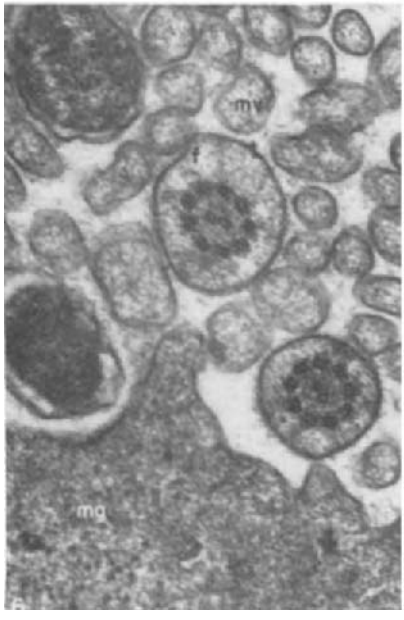
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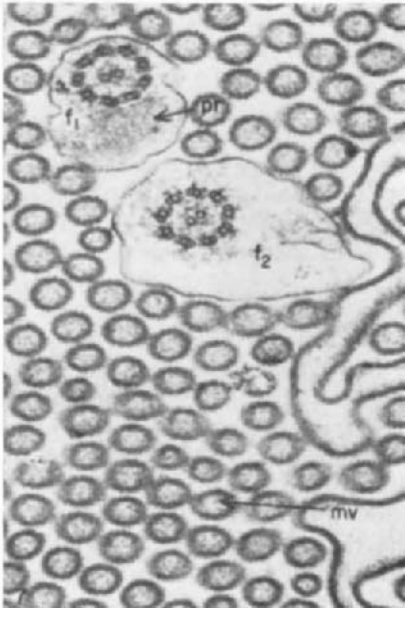
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evidence of genetic exchange. However, other enzyme markers are now available (Toyé, 1974) and such work should be followed up.

Brener (1973) has emphasised the need for a "critical re-examination" of *T.* (*S.*) *cruzi* in the invertebrate host. In addition the work of Lapierre (1972a) on the development and maintenance of *T.* (*S.*) *cruzi* in *Ornithodorus moubata* should be taken into account. Lapierre (1972a) found that *T.* (*S.*) *cruzi* developed in ticks infected with *Borrelia duttoni*. It is also necessary to utilise the large literature on *Rhodnius prolixus* physiology in conjunction with parasitological work, which will provide information relating to any physiological effect of *T.* (*S.*) *cruzi* on the vector, in the way Watkins (1971a,b) has done with *T.* (*H.*) *rangeli* in *R. prolixus*.

### B. SALIVARIA

The four subgenera of African pathogenic trypanosomes (*Duttonella*, *Nannomonas*, *Trypanozoon* and *Pycnomonas*) are discussed in this section in terms of their relation with their vectors, *Glossina*. The author has tried to review those aspects bearing on the detail of the vector–parasite relationship *sensu stricto*. The factors relating to infections with salivarian species in the vector have been included in tabular form (Table VI), as it was felt to omit reference to exogenous factors relating to mammalian host and parasite would give an incomplete picture. The nomenclature of the subgenus *Trypanozoon* suggested by Ormerod (1967b) is followed.

It should be mentioned that much work has been completed on aspects of salivarian trypanosomes which should be referred to here in a general introduction to the group although it does not form an integral part of the subject matter.

(1) The transmission of salivarian trypanosomes by mechanical means by flies of other genera as well as *Glossina* itself should be remembered as an important part of the epidemiology and epizootiology of these infections. Jordan (1974) reviews the current thinking in this subject and gives references to reviews of this topic.

FIG. 5. Section of midgut of *Melphagus ovinus* infected with *T.* (*M.*) *melophagium* and Mycoplasma-like organisms (**mlo**). Microvilli (**mv**, arrowed) of midgut cells have flagella (**f**) and anterior part (**ae**) of parasites inserted between them. Mycoplasma-like organisms (**mlo**) lie between microvilli and parasites. × 30 000.

FIG. 6. Higher magnification of same subject as Fig. 5, showing that flagella come into close contact with the midgut cell wall. Midgut cell (**mg**), other abbreviations the same. No hemidesmosomes are formed between flagella and microvilli and there is no expansion of the intraflagellar region. × 50 000.

(Figures 5 and 6 from Molyneux, 1975; reproduced by kind permission of the Editors of *Acta trop.*)

FIG. 7. Flagella (**f**) of nectomonads of *Leishmania mexicana amazonensis* interdigitated between microvilli (**mv**, arrowed) of midgut of *Lutzomyia longipalpis*. Note no hemidesmosomes between flagella and microvilli, and one of the flagella ($\mathbf{f_2}$) has no paraxial rod (**pr**, arrowed). × 50 000. (From Killick-Kendrick *et al.*, 1974; reproduced by kind permission of the Editors of *Proc. R. Soc. Lond.*)

(2) Much information is available on overall infection rates in wild caught *Glossina* and their parasites. This information is of local significance, and Jordan (1974) tabulated the recent information and discussed the shortcomings of the dissection techniques and identification methods of parasites in *Glossina*.

TABLE VI

*Factors influencing development of salivarian trypanosomes in* Glossina *and references to work associated with those factors*

| | |
|---|---|
| **Endogenous factors** | |
| 1. Fly age at infective feed | Wijers (1958); Harley (1971b) |
| 2. Sex | Duke (1933); Burtt (1946a); Fairbairn and Culwick (1950); Baker and Robertson (1957) |
| 3. Genetic differences between fly species | Harley (1971a); Duke (1933); Harley and Wilson (1968); Roberts and Gray (1972); Wilson *et al.* (1972); Janssen and Wijers (1974) |
| 4. Behaviour, i.e. host preference | Jordan (1965, 1974); Moloo (1973); Allsop *et al.* (1972) |
| 5. Physiological and biochemical state | Harmsen (1970, 1973) |
| 6. Virus, bacteria, symbiote infection in fly | Jadin *et al.* (1966); Jadin (1967); Jenni (1973); Jenni and Steiger (1974); Wei-Chun Ma and Denlinger (1974); Huebenr and Davey (1974); Pinnock and Hess (1974); Reinhardt *et al.* (1972) |
| **Ecological factors** | |
| 7. Climatic factors | Kinghorn and Yorke (1912); Kinghorn *et al.* (1913); Taylor (1932); Duke (1933); Burtt (1946a); Fairbairn and Culwick (1950); Fairbairn and Watson (1955); Ford and Leggate (1961); Jordan (1974) |
| 8. Available infected hosts | Fairbairn and Burtt (1946); Geigy *et al.* (1971) |
| 9. Hosts available for subsequent feeds | Duke (1935a); Van Vegten (1971) |
| **Parasite and hosts** | |
| 10. Parasite numbers available to fly | Van Hoof (1947); Baker and Robertson (1957); Page (1972b) |
| 11. Type of parasite (e.g. stumpy, slender); immune state of host | Robertson (1913); Wijers and Willett (1960); Baker and Robertson (1957) |
| 12. Subspecies; strain | Duke (1933, 1934); Van Hoof *et al.* (1938); Baker and Robertson (1957) |
| 13. Susceptibility | Duke (1935b); Ashcroft *et al.* (1959) |
| 14. Intercurrent infections | Willett (1972) |
| 15. Behaviour and attractiveness to fly | Corson (1935, 1936); Duke (1935b); Van Hoof (1947); Geigy *et al.* (1971); Allsop (1972); Allsop *et al.* (1972) |

(3) The definitive correlation between overall infection rates in *Glossina* and hosts. Jordan (1965) has shown there to be a positive correlation between trypanosome infection rates and the percentage of bloodmeals derived from Bovidae in Nigeria, and Moloo (1973) found a similar relationship between *G. swynnertoni* and Bovidae in the Serengeti, Tanzania. Both these authors reported that when meals that were taken from Suidae increased, infections with *Duttonella* decreased but *Nannomonas* infections were the same in flies whether they had fed on Suidae or Bovidae.

1. Subgenus *Trypanozoon*

The vast amount of literature available on this subject has made selection of material for the following section difficult. This is particularly so in view of the large amount of recent work which has been completed by various workers who summarise and review a great variety of branches of this subject (Mulligan, 1970; Hoare, 1972; Jordan, 1974). An attempt has been made to discuss in detail only those factors intrinsic to the vector which affect the relationship between parasite and host. However, reference has been made inevitably to other factors which have a bearing on infection rates in *Glossina* infected with *Trypanozoon* (see Table VI). The morphology of *Trypanozoon* in the vector has been described by Hoare (1972) and the fine structural morphology by Steiger (1973).

(a) *The peritrophic membrane and establishment barrier.* The initial studies on the structure and formation were made by Wigglesworth (1929), and a study of the relation of *T. grayi* to the peritrophic membrane of *Glossina fuscipes* was made by Hoare (1931a). Two years later Yorke *et al.* (1933) reported that trypanosomes passed round the broken posterior end of the peritrophic membrane of *Glossina*, multiplied in the ectoperitrophic space and then crossed the membrane anteriorly to the proventricular lumen. Yorke *et al.* (1933) believed that the peritrophic membrane anteriorly was of a more fluid consistency (3–4 $\mu$m thick), favouring penetration by the flagellates. Fairbairn (1958) similarly observed parasites in the membrane passing to the endoperitrophic space.

The importance of the peritrophic membrane in determining infection rates of *T.* (*T.*) *brucei* in *Glossina* was first hinted at by Buxton (1955), who stated "insects in the first and second day, taking their first meal, are much more easily infected by a trypanosome of the *brucei* subgroup than old tsetse. It is not known whether the difference is due to some biochemical condition . . . *or to some event in the development of the peritrophic membrane*" (my italics). Wijers' (1958) study on *T.* (*T.*) *b. gambiense* from monkeys in *G. palpalis* showed that flies fed within 24 h of emergence became infected much more easily than those fed later. This first day factor readily explained the observations of Duke (1935b) that multiple feeds on infected animals did not increase the infection rate. However, Duke himself considered that preliminary feeds on clean animals did not in fact make any apparent difference to the eventual number of infected flies—a view which was not shared by Van Hoof *et al.*

(1937). Wijers (1958) did not commit himself in his discussion but suggested that differences in the peritrophic membrane at an early age may be important in determining infection rates because being "weaker or looser . . . than subsequently", it may be easier to penetrate. Willett (1966) studied the peritrophic membrane of unfed flies. He showed this to be a complete sac and suggested that trypanosomes penetrated the soft semi-liquid part of the peritrophic membrane which in very young flies is an unusually high proportion of the membrane. In mature flies the soft part is much shorter as the growth rate is slower, hence penetration to the ectoperitrophic space is very uncommon. The ability of *G. austeni* to become infected more easily with *T.* (*T.*) *brucei* infections has been reported recently by Ward (1968). These flies were laboratory reared specimens and it is of interest to note that such effects first observed in the field are perpetuated in laboratory reared *Glossina*. However, Harley (1971b) showed that in *G. fuscipes* infected with *T.* (*T.*) *b. rhodesiense* the age of the fly at the time of the infecting feed is rather less important than might be expected from the results of Wijers (1958). Although flies 1–2 days old are much more susceptible, older ones *are* susceptible and can develop mature infections. There is little difference in susceptibility of flies between 2 and 11 days old. Earlier observations of a preliminary nature by Harley (1967) with *G. morsitans* and *T.* (*T.*) *b. rhodesiense* had indicated similar results.

Recently the invasion of the ectoperitrophic space by trypanosomes has been further studied by sections with the light microscope (Freeman, 1970, 1973). Freeman postulates that the explanation of trypanosomes being present in the ectoperitrophic space within 30 min of the infected bloodmeal being taken is that the trypanosomes penetrated the anterior end where it is freshly secreted. The other ways, (a) through the ruptured membrane after engorgement, or (b) by the classical route of travelling posteriorly to the open distal end and then migrating anteriorly, are dismissed. Theory (a) is rejected as both Hoare (1931a) and Willett (1966) found the peritrophic membrane intact after the feed, and (b) is rejected as the hindgut contents are lethal to trypanosomes as the pH is too low (5·8) (Bursell and Berridge, 1962). However, Ward (1968) has observed *T.* (*T.*) *brucei* occasionally in the hindgut of *G. austeni*, which he thought may have been due to contamination during dissection, and suggests a histological approach to this problem, stating that in an abnormal host–parasite system polymorphic trypanosomes may rarely undergo multiplication in the hindgut. It is perhaps worth recalling here that *T. grayi* survives particularly well in the hindgut of *Glossina*.

The higher infection rates obtained in first-day-fed flies (Wijers, 1958; Ward, 1968) explain the observation of Burtt (1946a) that flies kept at elevated pupal temperatures developed much heavier infection rates compared with control flies. This is likely to be due to the depletion of the flies' reserves by these elevated temperatures, thus necessitating an earlier feed for survival and thereby increasing the chances of becoming infected.

The low percentage of natural and experimental infections of *brucei* group trypanosomes in *Glossina* suggested to Harmsen (1973) that a barrier to infection of adult *Glossina* exists which, in view of the work of Wijers (1958) and Harley (1971a,b), is not fully developed until approximately 24 h after

emergence. Those flies which feed before this barrier falls will develop infections if infective trypanosomes are present in the meal. Harmsen studied this phenomenon experimentally in *G. pallidipes* infected with a strain of *T.* (*T.*) *brucei* ssp. as he felt that what is referred to as a minor mechanical hurdle could not itself be overcome by the parasite. Harmsen studied the distribution and movement of the blood in flies fed at different times and the growth of the peritrophic membrane by thick (100 $\mu$m) sections of entire flies which had been frozen. The peritrophic membrane grew at around 1 mm/h for 30 h but the rate gradually declined thereafter. The growth rate was higher at 25°C than at 30°C. If between 30 and 80 h the fly was fed, the original growth rate of 1 mm/h of the peritrophic membrane was resumed. Sections of flies interrupted during feeding showed that a small amount of blood went straight to the dorsal uncoiled midgut where it was contained in the membrane. The remainder of the blood filled the crop. Harmsen suggested that the amount of blood entering the midgut itself depended on the peritrophic membrane length, as it was never broken by overfilling. In flies over 30 h old the peritrophic membrane has extended into the second coil of the midgut, which can take almost the whole meal so that little goes into the crop, and what blood does go in, is transferred within 20 min. In flies fed early, transfer of blood from the crop can only occur after blood in the midgut is dehydrated and the peritrophic membrane has grown; this may take 5–6 h.

In the light of these observations infection of the ectoperitrophic space by the following methods can be ruled out. As blood never penetrates a ruptured membrane (Freeman, 1973), and in younger flies the membrane does not grow faster when blood is transferred from the crop to the midgut, it seems that easier penetration at this stage is unlikely. In flies fed at 60 h the blood will reach to the posterior end of the midgut but even so the membrane remains intact. Harmsen (1973) pointed out that the crop emptying rate is very different between young and old flies. In young flies the blood is retained in the crop for 45–75 min, but 24 h after emergence the length of time the meal remains in the crop is much reduced (see Fig. 3 in Harmsen, 1973). Harmsen suggested that two possibilities exist relating infectivity to crop emptying rate: (1) that the slow flow of blood from the crop enables penetration of the soft part of the peritrophic membrane to occur more easily or (2) that the length of time spent in the crop enables the parasites to adapt so they are more suitable for survival in the fly. The evidence for the first hypothesis has been strengthened to some extent by Freeman's (1973) observation, but Harmsen's evidence for the second idea is based on his finding that trypanosome glucose-6-phosphatase activity levels, properties and localisation changed after 1 h in the crop. Harmsen concludes from this that in addition to the change which the stumpy forms have undergone in the mammalian bloodstream, preadapting to life in *Glossina* (Vickerman, 1965), an additional period of 45–60 min adjustment is required in the crop to enable trypanosomes to survive in the midgut. This can only happen in flies with a poorly developed peritrophic membrane. In quantitative terms this is 24 mm long and it is at this length 24 h after emergence; flies which feed within this period shunt most of the blood meal to the crop for 60 min whilst the peritrophic membrane grows and the initial blood volume

is reduced by water resorption. The parasites in the crop blood can change in this time to become better adapted biochemically to the midgut. Harmsen (1973) discussed the weaknesses of this argument and admits that the mechanism of invasion of the ectoperitrophic space is still not accounted for; the ideas of Willett (1966) and Freeman (1973) seem most likely to explain this. However, the infection rates, although much higher in flies fed in the first 14 h after emergence compared with older flies, are still very low. This indicates a second barrier which possibly prevents development of mature infections even though the first barrier is overcome and established infections are initiated (Dipeolu and Adam, 1974).

Clearly the ideas of Harmsen (1973) represent a new experimental approach to the fascinating problem of *T.* (*T.*) *brucei* in *Glossina*. Much work needs still to be done to unravel this complex cycle, which has attracted so much attention but which has revealed so few secrets over nearly 70 years.

(b) *Haemocoelic infections.* Although Foster (1963, 1964) recorded haemocoelic infections of *Glossina* in Liberia, these parasites did not appear to be *brucei* group trypanosomes. Mshelbwala (1972) has reported *T.* (*T.*) *brucei* in the haemocoele of experimentally infected *Glossina*. Out of a total of 1285 flies dissected by Mshelbwala, 150 (11·58%) had mature salivary gland infections and 112 (8·65%) had immature infections. Of these 262 infected flies, 40 (15·27%) had trypanosomes in the haemocoele. The infections were detected by examining the minute drop of fluid extruded after cutting off the legs and wings of the flies. Three species of *Glossina* were used, *tachinoides*, *morsitans* and *palpalis*, and haemocoelic infections were observed in all three species. The morphology of the parasites varied from midgut trypomastigote forms, through epimastigotes to typical metacyclic forms. In addition to these characteristic forms, amastigotes were also found. Trypanosomes from the haemocoele of some flies when inoculated into mice produced typical *T.* (*T.*) *brucei* infections. Otieno (1973) has now confirmed these findings. He found haemocoelic infections in *G. morsitans* only 3 days after the infecting feed—Mshelbwala (1972) saw haemolymph infections only after the tenth day of the infection. Otieno (1973) probably also used an East African strain of *T.* (*T.*) *brucei*, although this is unspecified, but confirmation of haemolymph infections in *Glossina* is gratifying, for Mshelbwala suggested that it may be a particular characteristic of his own strain of *T.* (*T.*) *brucei*. The trypanosomes in the haemolymph have probably originated from the gut. Penetration of the midgut cells of *G. m. morsitans* by *T.* (*T.*) *b. rhodesiense* has been demonstrated by Evans and Ellis (1975) using flies fed on cultured midgut forms. Parasites were found also between the midgut cell basement membrane and the haemocoele membrane. No pathogenicity was observed as a result of the infection. The significance of these infections must await further work to determine those factors influencing invasion of the haemocoele and, if it occurs in *T.* (*N.*) *congolense* and *T.* (*D.*) *vivax*, to determine whether parasites can invade the salivary glands from the haemocoele and whether the parasites can invade the proboscis from the bathing haemocoelic fluid.

(c) *Ultrastructure of* Trypanozoon *in relation to* Glossina. Vickerman and Perry (1968) have described briefly the relation between the peritrophic membrane and *T.* (*T.*) *b. rhodesiense*, but no micrographs were published. These authors reported that parasites became sandwiched between the peritrophic membrane and the invagination of the foregut, and embedded parasites moved posteriorly until they escaped from the peritrophic membrane and reached the endoperitrophic space. As parasites were not found embedded in the gut further posteriorly it seemed they extricated themselves easily. It was also noted that the parasites in the peritrophic membrane of *Glossina* were well fixed and were assumed to be healthy at the time of fixation. Vickerman (1969) has described the structure of the metacyclics of *T.* (*T.*) *b. rhodesiense* in *Glossina* salivary glands but did not discuss the relationship between the parasite and salivary gland epithelium.

Steiger (1973), however, has described the relationship between the various stages of *T.* (*T.*) *brucei* infection in the fly and the surfaces and structures with which they become associated, as well as the detailed ultrastructure of all these stages. The established trypomastigote midgut forms are found within the ectoperitrophic space, and Steiger showed these forms to be different structurally from those found further forward in the proventricular region. The parasites are found between the peritrophic membrane and the microvilli lining the endodermal epithelial cells. Rarely do the flagella of the parasites penetrate between the microvilli as do other flagellates in the midgut (e.g. *Leishmania*, see Killick-Kendrick *et al.*, 1974; *T.* (*M.*) *melophagium*, see Molyneux, 1975). Some strains of *T.* (*T.*) *brucei*, however, have apparently a more close affinity with these cells penetrating between the microvilli (Steiger, personal communication). Trypanosomes become embedded in the peritrophic membrane, the ultrastructure of which has been described by Moloo *et al.* (1970). These authors recognised three cell types in the region of formation of the membrane which were responsible for the secretion of the different constituents of the membrane. The peritrophic membrane appears to be bilaminate, the first (outer) layer being of mucopolysaccharides, giving an electron-dense appearance, the second layer, which faces the microvilli, being proteinaceous in nature. Moloo *et al.* (1970) discussed the relation between the trypanosomes and the peritrophic membrane. They believed that the observation of Freeman (1970, 1973) of trypanosomes within the peritrophic membrane 30 min after a bloodmeal was incompatible with the membrane structure. They suggested that in fact the trypanosome seen by Freeman (1970) was in a fold of membrane and penetration at the position described by her would require some enzymatic mechanism to force it through the barrier. Moloo *et al.* (1970) suggested that the point of crossing the membrane barrier from ecto- to endoperitrophic space was in the region of the Type II cells where the outer layer is still not polymerised. Steiger (1973), however, has not confirmed this idea, finding that parasites were embedded between the two layers of peritrophic membrane and that the layers were pulled apart leaving a cavity with floccular material inside containing the parasites.

In the proventricular regions (between midgut cells and peritrophic membrane in front of the press) the parasites were closely pushed together. Emergence from

the ectoperitrophic space to the endoperitrophic space occurred in the region of the cells which secreted the thicker proteinaceous layer of the membrane. The area of penetration lay ahead of the press where the thickness of the membrane was between 0·5 and 3·5 $\mu$m but where the lamina was not yet fully polymerised. Steiger recognised three different steps in the penetration of the membrane: (1) trypanosomes embedded in the peritrophic membrane, (2) appearance of parasites at periphery of the outer layer where they lie in a "stratum-like distribution", (3) the release of the parasites into the endoperitrophic space of the proventricular region by the rupture of this outer layer. Steiger found that trypanosomes never lay perpendicularly to the peritrophic membrane but were oblique or horizontal in position in relation to the axis of the membrane (Fig. 9).

In the salivary glands of *Glossina*, Steiger (1973) found that the optimal parasite density occurred in the middle part of the glands which was lined with microvilli. The epimastigote forms were concentrated in the epithelial lining with the flagella penetrating deep into the microvilli. Thus the flagella function as an anchor. In addition to this, Steiger observed hemidesmosomes on the flagellar membrane side of the junction between salivary gland microvilli and flagella (Fig. 8). Metacyclic parasites were only observed in the lumen of the salivary gland and did not appear to be associated with host tissue.

(d) *Membrane feeding*. The first worker to infect *Glossina* with trypanosomes after feeding on blood and parasites through membranes was Mshelbwala (1967). Recently this technique has been exploited by Dipeolu and Adam (1974) as a means of obtaining standard infection in laboratory reared *G. morsitans*. By using material from animals whose parasitaemia was made up of antigenically similar organisms, flies could be infected at different times with identical parasite populations. As the only variable with this technique was the length of time the parasites had been cryopreserved, the effects of other variables could be better assessed. Dipeolu and Adam (1974) obtained mature salivary gland infections in these flies on 15 occasions although 39 flies transmitted the infection to mice. These authors recognised three types of infection in *Glossina*: initial, established and mature. Initial infections were represented in about 50% of flies at 3 days, when trypanosomes were present in the posterior midgut both within and outside the peritrophic membrane. Established infections occurred in about 9% of flies by the 5th day when parasites had migrated forward into the ectoperitrophic space of the middle and anterior parts of the midgut. The numbers of flies with established infections did not vary between 5–30 days after the infective feed. The penetration into the proventricular region always occurred in established infections. The above pattern of development is confirmation that the results of membrane feeding are similar to the classical early description of Robertson (1913), who dissected *G. palpalis* infected with *T.* (*T.*) *b. gambiense*. Dipeolu and Adam (1974) defined mature infections as those which would transmit the infection to mice. The results of the above authors when varying such experimental factors indicated that the properties of flies with initial infection varied depending on the temperature they were maintained at, the strain of trypanosomes and the number in the

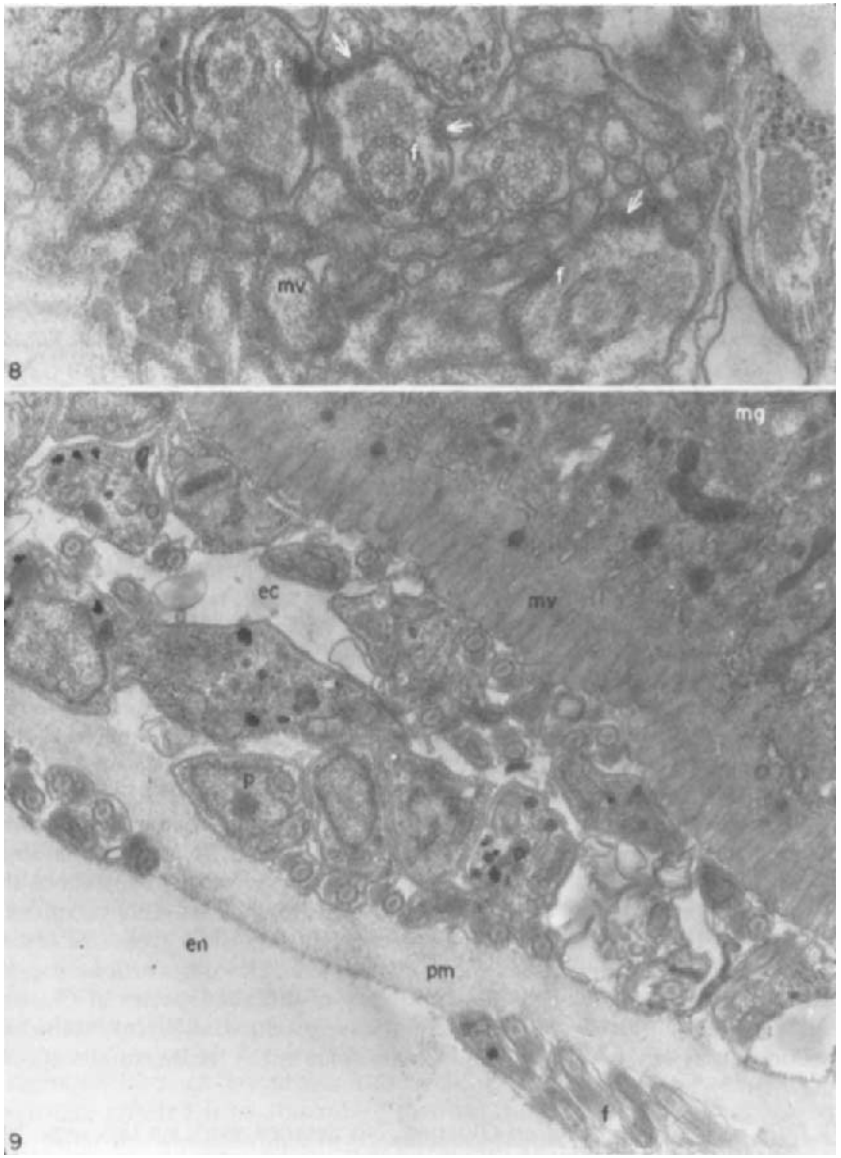


FIG. 8. Flagella (**f**) of epimastigote form of *T.* (*T.*) *brucei* inserted between microvilli (**mv**) brush border of *Glossina* salivary gland epithelium. Small arrows indicate hemidesmosomal plaques. × 172 000.

FIG. 9. Midgut forms of *T.* (*T.*) *brucei* in *Glossina* in ectoperitrophic space (**ec**) and endoperitrophic space (**en**). Peritrophic membrane (**pm**) lies between. Parasites (**p**) are packed between microvilli (**mv**) of midgut cell (**mg**). Flagella (**f**) are aggregated together, lying obliquely to peritrophic membrane in endoperitrophic space of proventricular region. × 14 800.

(Figures 8 and 9 from Steiger, 1973; reproduced by kind permission of the author and of the Editors of *Acta trop.*)

infecting feed. Those with mature infections varied according to the temperature of fly maintenance and pupal incubation. None of the tested variables significantly altered the numbers of flies with mature infections, the proportion of which is suggested by Dipeolu and Adam (1974) to be a feature of the colony of *G. morsitans* used.

Dipeolu and Adam (1974) found that the proportion of flies which had established infections and which became mature, varied considerably. Special conditions may be required for the final phase of development. What these conditions are must remain a matter for speculation, but the ideas put forward by Harmsen (1970) are worth considering. He found pteridines which have been noted to have a marked effect on growth of *C. fasciculata* in *Drosophila* (see review by Zeigler and Harmsen, 1969; see also Section VA) to be present in measurable amounts concentrated in the eyes of *G. pallidipes*, and suggested that although the availability of such substances to trypanosomes is unknown they may exert an effect on parasite migration and morphogenesis. This is possibly more significant now as a result of the finding of haemocoelic infection of *T. (T.) brucei* in *Glossina* (Mshelbwala, 1972), as these compounds may be circulating in the haemolymph and determining trypanosome distribution and growth rates.

(e) *Transmission of* Trypanozoon. The presence of salivary gland infections has for some time been in doubt as a true indication of the infection rate of *Glossina* with trypanosomes of the subgenus *Trypanozoon*. Recent studies (Ward and Bell, 1971; Page, 1972b; Dipeolu and Adam, 1974) have shown that more flies can transmit infections to mice than the number of that particular batch with salivary gland infections.

Other workers have dissected thousands of flies in areas of heavy *T. (T.) brucei* infection rates in mammals without finding a single salivary gland infection (see Rogers and Boreham, 1973; Moloo *et al.*, 1973). These findings must raise doubts (Dipeolu and Adam, 1974; Moloo *et al.* 1973) about the site of development of infective forms and about whether proventricular forms or forms in the proboscis, which cannot be distinguished from *Duttonella* and *Nannomonas* during dissection, are infective. The observations suggest also that comparative work on susceptibility of different species of *Glossina* (e.g. Harley, 1971a) to *Trypanozoon* might have produced different results had infection rates been judged not by dissection alone but by feeding on susceptible mice.

(f) *Effects of* Trypanozoon *on* Glossina. No detailed work on this topic has been published and certainly no ill-effects of *T. (T.) brucei* in *Glossina* have been found. Baker and Robertson (1957), however, reported that female flies with salivary gland infections of *Trypanozoon* appeared to have a greater longevity. It was not possible to confirm this statistically but further work on this subject with standard techniques and larger numbers of laboratory-reared flies may prove rewarding.

2. Subgenus *Nannomonas*

The biology of *T. (N.) congolense* and *T. (N.) simiae* has been reviewed by

Hoare (1970, 1972), who gives references to the early work on the life-cycle of *Nannomonas* species in the vector as well as details of the morphology of these parasites in the fly. Jordan (1974) has tabulated the infection rates of *Glossina* species infected with *Nannomonas* species from various areas. Only more recent work is included here.

(a) *T.* (*N.*) *congolense*. This parasite has been cyclically transmitted through *G. morsitans* and laboratory mice by Elce (1971, 1974), who found that 12–13% of flies became infected by 10–14 days although no fly became infected later than 17 days after taking the bloodmeal. Elce reported that one in five infected flies is capable of infecting mice and can do so at every meal. Individual flies, however, more frequently produce "sub-threshold" numbers of parasite and transmission is only assured when two or more feed simultaneously. One out of four flies remained infective for life, the remainder lost the infection after a small number of meals. These results indicated that *T.* (*N.*) *congolense* in mice can be used as a laboratory system for transmission through *Glossina*. Studies on the relationship between *T.* (*N.*) *congolense* and the midgut and proboscis have not yet been made as they have in *Duttonella* (see Vickerman, 1973). Such information will be of great interest.

Gray and Roberts (1971a,b) have shown that as with *T.* (*D.*) *vivax*, strains of *T.* (*N.*) *congolense* resistant to diminazene aceturate and homidium chloride can be transmitted through *G. morsitans* and *G. tachinoides* and retain the characteristic level of resistance on passage to susceptible hosts.

(b) *T.* (*N.*) *simiae*. Roberts (1971) has studied the behaviour of a strain of *T.* (*N.*) *simiae* from Nigeria in *G. m. submorsitans* and *G. tachinoides*; infection rates as revealed by dissection varied between 0 and 8·6% in *G. m. submorsitans* and 1·6%–6·3% in *G. tachinoides*. Cyclical development took place in 16–25 days.

Janssen and Wijers (1974) have recently reported that the virulence of strains of *T.* (*N.*) *simiae* from the Kenya coast was related to the species of fly through which they were passaged or from which they were isolated. Thirteen strains of *T.* (*N.*) *simiae* were isolated from *G. brevipalpis* which were very virulent in pigs, while a strain from *G. pallidipes* produced a chronic infection in pigs; two strains isolated from *G. austeni* were intermediate in character between the strains from *G. brevipalpis* and *G. pallidipes*. An acute strain which originated from *G. brevipalpis* was cyclically passaged to a rabbit and *G. pallidipes* infected from this rabbit then transmitted the infection back to a pig. After the passage through *G. pallidipes* the isolate had lost its virulence to the pig. This work indicates that the virulence of a trypanosome may be related to the species of fly with which it is associated. *G. pallidipes* also appears to be more resistant to infections with *T.* (*N.*) *simiae* compared with *G. brevipalpis*, as natural infection rates in the latter species are far higher, though this may of course be due to other factors (see Table VI). Further experiments should be initiated to confirm this interesting observation referred to above.

3. Subgenus *Duttonella*

The behaviour of *T.* (*D.*) *vivax* in *Glossina* has been reviewed by Hoare

(1972). The cycle of *T.* (*D.*) *vivax* in *Glossina palpalis* was first studied by Bruce *et al.* (1910, 1911), who established the basic facts of the life-cycle of this trypanosome in the fly. They observed that established infections of clusters of epimastigotes occurred only in the proboscis of this fly and that the infection rate in experimentally fed flies was 17%. Trypanosomes taken into the midgut of *G. palpalis* survived only 24 h according to Bruce *et al.* (1911), but Lloyd and Johnson (1924) found parasites in this site up to 4 days after a meal. Infection of the proboscis is apparently established directly the parasites pass through the food channel prior to being carried into the crop and midgut. Here attachment and subsequent transformation to epimastigotes takes place. The epimastigotes of *T.* (*D.*) *vivax* are attached to the cuticular lining of the labrum (Vickerman, 1973) by flagellar hemidesmosomes, the parasites being aligned in such a way that the flagellar axonemes run parallel to the length of the labrum and the parasites are swept posteriorly as blood rushes past during feeding. The parasite body never appears to be attached to the food canal. Vickerman (1973) showed that the attached flagella adhered to an encrustation of the tsetse labrum (Fig. 10). The flagellar membrane and labrum were separated by 12 nm and the hemidesmosomal plaque was found on the inner leaflet of the flagellar membrane. Filaments extended from the dense zone into the intraflagellar area. Where the membrane was separated from the labrum by more than about 12 nm the hemidesmosomal plaque was not observed. The flagella of other parasites in the clumps were attached to the adherent flagella by macular desmosomes which were similar to the structures attaching the flagellum to the parasite body at the undulating membrane.

Vickerman (1973) rarely encountered flagella associated with the parasite body of epimastigotes attached to the cuticle, and expressed the view that the arrangement of the attachment mechanisms and the parasite play an important role in the maintenance of the epimastigotes in their sites, presenting minimal resistance to the flow of blood. A study of the encrustation of the cuticle of the labrum was suggested by Vickerman (1973).

The maintenance of the flagellates in the type of clump described by Bruce *et al.* (1911) is dependent, however, not only on the zonular hemidesmosomes but also on the macular junctions between flagella. Vickerman (1973) stresses that these junctional complexes all require modification of material within the flagellar sheath, and the ability of the parasite to produce such changes at a particular time and in a particular site is a highly important adaptive survival mechanism. The structure of the metacyclic trypanosomes of *T.* (*D.*) *vivax* in the hypopharynx was not reported by Vickerman (1973) but it is reported that the epimastigotes detach from the proboscis clumps and infect the hypopharynx where preinfective trypomastigotes and metacyclics are produced. The times when metacyclics were found in the hypopharynx and could produce infective forms varied. Roubaud (1935) reported that they were within 48 h, Lloyd and Johnson (1924) after 13 days. Desowitz and Fairbairn (1955), however, found that development to metacyclics took between 12–13 days at 22°C and 5 days at 29°C. Bruce *et al.* (1911) reported that in *Glossina* infected with *T.* (*D.*) *vivax* the infection persisted for 58 days.

Those species of *Glossina* which have been found to be vectors of *T.* (*D.*) *vivax*

have been listed by Hoare (1972). The infection rate of *Glossina* with *T.* (*D.*) *vivax* is recognised to be higher than that of the other salivarian subgenera *Trypanozoon*, *Pycnomonas* and *Nannomonas*. The infection rates obtained vary according to author, area, fly species and strain of parasite (Jordan, 1974).

Gray and Roberts (1971a,b) have shown that *T.* (*D.*) *vivax* strains which show resistance to diminazene aceturate and quinapyramine sulphate retain

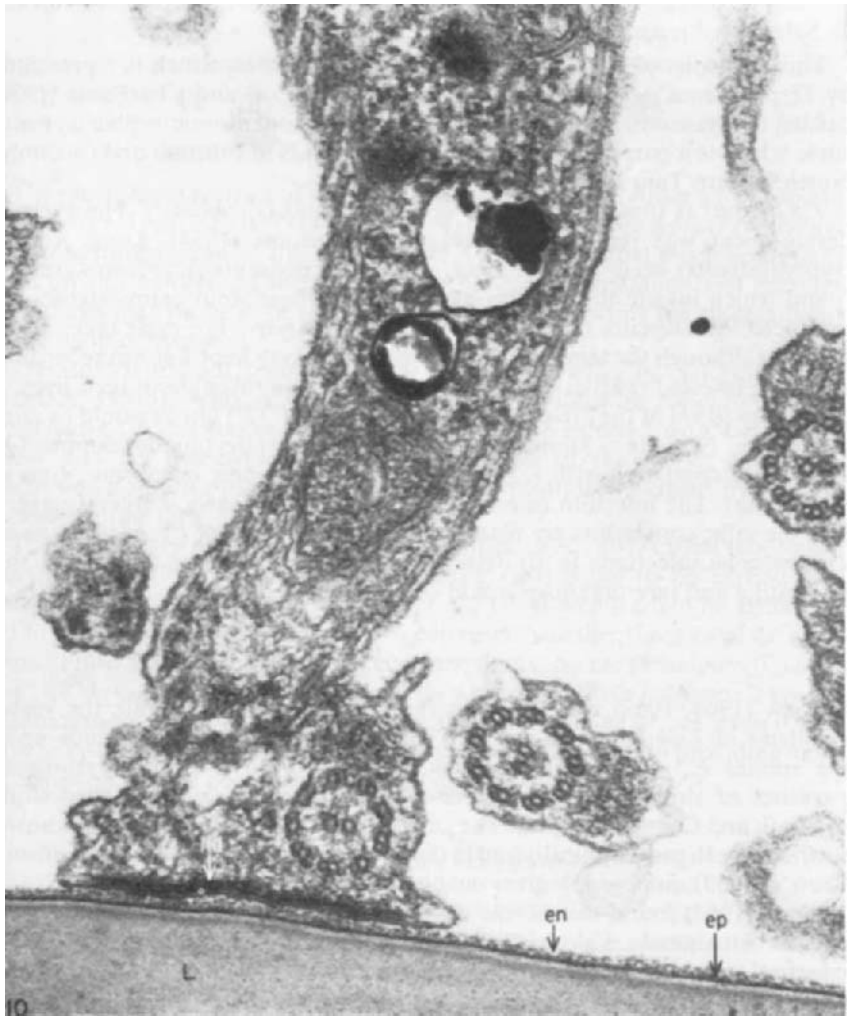


FIG. 10. *T.* (*D.*) *vivax* attached to the labrum (**L**) of *G. fuscipes*. Hemidesmosome (**hd**, arrowed) within intraflagellar region. The flagellar membrane is closely associated with an encrustation (**en**, arrowed) which lies on the thin dark line of epicuticle (**ep**, arrowed). × 68 000. (Reproduced by kind permission of Professor K. Vickerman.)

*their ability to transform and develop cyclically in Glossina palpalis.* These results indicate that the transmissibility and behaviour of *T. (D.) vivax* in *Glossina* does not differ between drug-resistant and non-resistant strains.

*T. (D.) vivax* occurs outside Africa where *Glossina* species are not found (Hoare, 1972; Lainson and Shaw, 1972a). This indicates that transmission by mechanical methods probably occurs. However, the patterns of infection observed suggest that cyclical development in an insect other than *Glossina* may occur (Wells, 1972b; Page, 1972a).

4. Subgenus *Pycnomonas*

This subgenus of *Glossina*-transmitted trypanosomes, which is represented by *Trypanosoma suis*, has not been studied since Peel and Chardome (1954) carried out transmission studies on *T. (P.) suis*, a stout monomorphic trypanosome which is a parasite of wild and domestic suids in Burundi and (possibly) North Eastern Tanzania.

*T. (P.) suis* is transmitted by *G. brevipalpis* and *G. vanhoofi*. The cycle of development was reported by Peel and Chardome (1954). Long, slender trypomastigotes occur in the midgut, and slender proventricular forms are then found which invade the salivary glands where short stout epimastigotes are produced. Metacyclics then occur in the hypopharynx. The cycle takes about 28 days (although the temperatures at which flies were kept was not recorded), and once infected the flies can transmit the infection throughout their lives.

A comparison of the life-cycle of *T. (P.) suis* and *T. (T.) brucei* would be most interesting. Nothing is known regarding the factors affecting development of *T. (P.) suis* compared with *T. (T.) brucei* (e.g. peritrophic membrane, time of fly feeding). The infection rate of 1%–2% quoted by early workers suggests that the same constraints are placed on the development of *Pycnomonas* as on *Trypanozoon* infections in *Glossina*. Further research on all aspects of this interesting and rare organism would be welcomed.

### C. GENUS *ENDOTRYPANUM*

Shaw (1964, 1969) was the most recent worker to investigate the vector problems of *Endotrypanum* species. The genus *Endotrypanum* is made up of the species *E. schaudinni* and *E. monterogeii;* both are intra-erythrocytic parasites of sloths (*Bradypus*, three-toed sloth; *Choloepus*, two-toed sloth) of South and Central America. The genus *Endotrypanum* is, so far as is known, restricted both geographically and in the hosts which it is capable of parasitising. Shaw's (1969) monograph gives details of knowledge of this genus.

Shaw (1964) found that sloths were frequently fed on by *Lutzomyia* when used as bait animals. A sloth (*Choloepus h. hoffmani*) infected with *E. schaudinni* was fed on by *Lu. trapidoi* (Shaw, 1969). Dissection of these flies revealed initial multiplication of flagellates in the bloodmeal with the appearance of syncytial amastigotes with a lobulate structure after the parasites had escaped from the sloth erythrocytes. When the bloodmeal was voided haptomonad and nectomonad promastigotes were observed in the midgut and, as small colonies of rosettes, attached in the hindgut on the 6th day after feeding,

although no parasites were observed in the hindgut triangle or rectum. The ability of the parasites to establish suggested to Shaw that *Lutzomyia* was the likely vector of *Endotrypanum*, despite the fact that early attempts to infect laboratory-reared *Lu. sanguinaria* with *E. monterogeii* from sloths and by artificial feeding of culture forms in a Hertig apparatus were unsuccessful. The ability of the parasite to attach suggested that *Lutzomyia* were likely vectors, on the basis that attachment of the parasites plays an important role in the vector efficiency of the parasite–vector combination. The method of transmission of *Endotrypanum* to sloths is not yet known, however.

## IV. *LEISHMANIA*–SANDFLY RELATIONSHIPS

### A. LIZARD *LEISHMANIA*

The most recent reviews of sandfly–*Leishmania* biology (Adler and Theodor, 1957; Adler, 1964; Lewis, 1974; Bray, 1974) have listed those workers who have produced information on this topic. There is little doubt that much needs to be done on this subject, as the relationship between mammalian and reptilian *Leishmania* may prove to be of importance in understanding the epidemiology of the human disease.

The only work not reported in the above reviews is by Saf'janova and Alexseev (1975), who have reported that *Phlebotomus papatasi* and *S. arpaklensis* (= *Sergentomyia antennata*) could be infected with *Le. tropica major* and *Le. gymnodactyli* by a modification of the Hertig apparatus. There was no indication given on the relative positions assumed by the two parasites in the flies.

The availability of sandflies (Killick-Kendrick *et al.*, 1973) from laboratory colonies will allow further work on reptile *Leishmania* to be carried out. Several species are available (Chance *et al.*, 1974) which could be artificially fed to sandflies by Hertig apparatus to determine whether the sites of development fit into a distinctive pattern. Biochemical taxonomic techniques (Chance *et al.*, 1974; Gardener *et al.*, 1974) have shown that reptilian *Leishmania* are not a homogenous group—initial studies of the type outlined above, even if they have to be made in a New World sandfly, may prove valuable in providing more information on this host–parasite relationship.

### B. MAMMALIAN *LEISHMANIA*

#### 1. *Life history and morphology of* Leishmania *in sandflies*

The transformation from the mammalian form, the amastigote, to the typical invertebrate form, the promastigote, and the subsequent development and migration of the parasites, have been described by several authors using the light microscope for *Le. donovani* in *Phlebotomus argentipes* (Knowles *et al.*, 1924; Christophers *et al.*, 1925; Shortt *et al.*, 1926; Shortt, 1928), for various Mediterranean isolates (see Adler and Theodor, 1957) and for the South American isolates (see review of Lainson and Shaw, 1974). A similar type of

development occurs in all mammalian *Leishmania* in sandflies apart from those members of the *Le. braziliensis* complex where forms are found in the hindgut and hindgut triangle. Initial development transformation and multiplication occur in the stomach. The forms found in this region are long slender active promastigotes, termed nectomonads, a term resurrected by Killick-Kendrick *et al.* (1974): as the peritrophic membrane breaks up the parasites migrate forward into the anterior midgut and cardia or, in the case of the *Le. braziliensis* complex, to the hindgut and hindgut triangle where short attached forms are observed, the significance of which is not known. However, in the *Le. braziliensis* complex anterior development is seen similar to that of the other types of parasite (Lainson *et al.*, 1973). Transformation to shorter forms attached to the oesophageal valve, termed haptomonads, is found around the third day after the infective feed. In susceptible sandflies fed on *Leishmania* development proceeds as far as this in a majority of cases, and blocked anterior midgut, cardia and oesophageal valve infections are frequently encountered. However, the factors which determine further anterior migration to oesophagus, pharynx, buccal cavity (cibarium) and proboscis are unknown, as the proportion of infections located in these sites is far smaller than with oesophageal valve infection (Adler and Ber, 1941). There are several references in the literature to short rounded forms being present in the pharynx of flies infected with various *Leishmania* (Adler and Theodor, 1935; Adler *et al.*, 1938; Adler and Theodor, 1957; Adler, 1963; Coelho *et al.*, 1967b,c). Adler (1963) believed that the "short" forms indicated "differentiation towards forms which are more easily transmissible by bite . . . with a flagellum longer than the body . . .. These forms appear to invade the proboscis more readily than others but they are often absent." These observations are particularly interesting in the light of the observations of pharyngeal forms of *Le. mexicana amazonensis* in the sandfly *Lutzomyia longipalpis*, for Molyneux *et al.* (1975b) found such smaller irregularly shaped organisms with an opisthomastigote configuration attached to the cuticle wall of the pharynx by flagellar hemidesmosomes. Although the significance of these forms is still unproven there is a temptation to regard them as a terminal infective stage of a developmental cycle from long slender nectomonads through attached haptomonads to opisthomastigotes.

Killick-Kendrick *et al.* (1974) and Molyneux *et al.* (1975a,b) have also investigated various aspects of the ultrastructure of *Le. m. amazonensis* in *Lu. longipalpis* bred in a laboratory colony. Although it is realised by these workers that in natural conditions *Lu. longipalpis* does not transmit *Le. m. amazonensis*, and that it is often not possible to reproduce the physiological status of wild sandflies in laboratory bred ones, and that such findings must be interpreted with caution (Adler, 1963), they nevertheless feel that such a system is useful. This is particularly so as *Lu. longipalpis* can transmit this infection by bite to hamsters.

Killick-Kendrick *et al.* (1974) described the modifications of the flagella of the nectomonads and haptomonads associated with the various surfaces of the gut. In the first 48 h after the infective feed the parasites were found in the blood and were retained within the peritrophic membrane. Some parasites were observed embedded in this structure during this time and remained there

as it broke up and the bloodmeal was digested and passed out. The break-up of the peritrophic membrane resulted in the parasites, which had multiplied and transformed to nectomonads, being capable of colonising the remainder of the midgut. At this stage the microvilli lining the midgut wall elongated and sections of the parasitised midgut revealed that unmodified flagella were inserted deeply into these structures. As early as 1926 Adler and Theodor and later Perfil'ev (1966) observed that the flagella lay entangled among the rods projecting from the epithelial cells. Killick-Kendrick *et al.* (1974) found that no hemidesmosomes were found between the microvilli of the midgut and the flagella despite a very close association (Fig. 7). The flagella of some of the nectomonads were modified and myelin figures were formed within the flagellar membrane; multiple axonemal structures were also found. The former process was believed to be associated with flagellar shortening associated with the morphological change to haptomonad forms in the cardia and oesophageal valve. In those regions of the midgut where degenerating epithelial cells were observed with no microvilli bordering the gut lumen, flagella have been observed to penetrate into the cytoplasm of the cells through tunnels (Killick-Kendrick *et al.*, 1974; Molyneux *et al.*, 1975a,b).

There is a region of sharp transformation in the anterior midgut between nectomonads and haptomonads, the latter being packed into the cardia. Flagella were not observed between the closely packed microvilli in this area but the flagella of the haptomonads were found to be attached to the oesophageal valve by flagellar hemidesmosomes. The oesophageal valve is a cuticle-lined structure which juts into the cardia and light microscope sections revealed the haptomonads to be attached to the surface. Adler (1963) observed that the distal end of the flagellum of these forms was wider and that in those species of *Phlebotomus* which were specific vectors attachment occurred to this site. The ultrastructure of this attachment (Killick-Kendrick *et al.*, 1974) showed that the intraflagellar area of the haptomonads was expanded and might contain myelin figures but distally the flagellar membrane was lined with an electron-dense hemidesmosomal plaque at those points where the flagella were attached to the oesophageal valve cuticle. Flagella of parasites in close proximity to the oesophageal valve but in contact with microvilli did not exhibit hemidesmosomes (Fig. 12). The flagellar membranes became moulded to knobs and protrusions of cuticle. The hemidesmosomes measured 40–55 nm in thickness and the distance between the flagellar membranes and the cuticle was 20–25 nm where hemidesmosomes were formed. No hemidesmosomes were observed where the distance separating the two surfaces exceeded 30 nm, a similar initial distance to that observed by Brooker (1970) in a study of desmosomes of *Crithidia fasciculata* in culture (see Section VA). Material was observed to stream forward to the hemidesmosome from within the intraflagellar area. As mentioned above, similar hemidesmosomal junctions were found between the flagella of opisthomastigote-like parasites and the pharynx cuticular epithelium (Molyneux *et al.*, 1975b).

The production of hemidesmosomes may be part of the mechanism which determines vector–parasite specificity. As establishment on the oesophageal valve seems to be a prerequisite for development in more anterior regions of

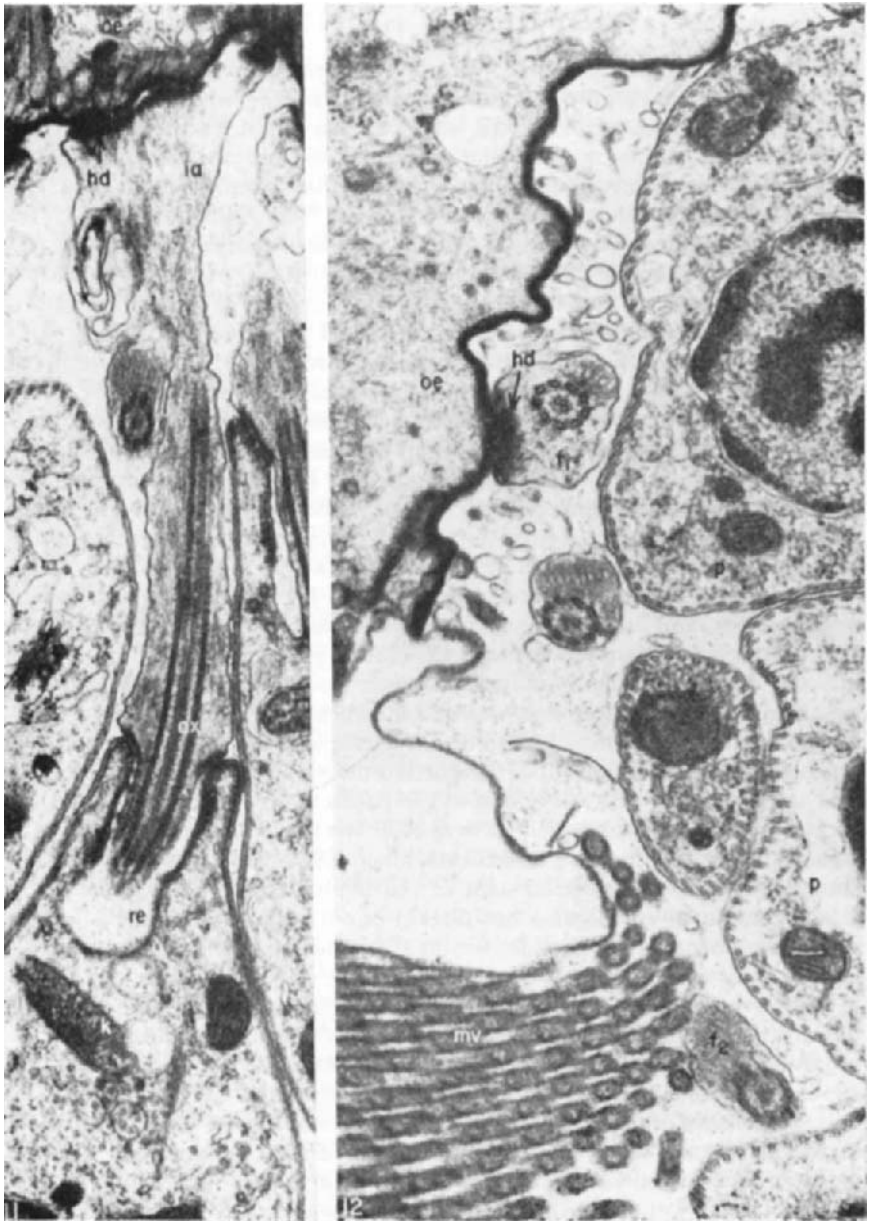


FIG. 11. Section through a haptomonad of *Leishmania mexicana amazonensis* attached to oesophageal valve (**oe**) cuticle of the sandfly *Lutzomyia longipalpis*. Hemidesmosome (**hd**, arrowed) formed within enlarged intraflagellar area (**ia**). Axoneme (**ax**); reservoir (**re**); kinetoplast (**k**). × 30 000.

FIG. 12. Section of junction between oesophageal valve (**oe**) of sandfly (parasite and fly as above) and microvilli (**mv**) lined midgut. Note flagellum ($f_1$) attached to oesophageal valve has hemidesmosome (**hd**, arrowed), whereas that associated with microvilli (**mv**) ($f_2$) has not. Parasites (**p**). × 30 000.

(From Killick-Kendrick *et al.*, 1974; reproduced by kind permission of the Editors of *Proc. R. Soc. Lond.*)

the fly, those factors which stimulate further anterior migration are likely to be of great importance if transmission is to take place.

2. *Transmission*

Although it was known in 1924 that a cycle of development occurred in sandflies, it was not for a further 18 years that transmission of the parasite by bite was obtained. The steps in the achievement of this breakthrough are described by Adler and Theodor (1957). As described below it was the inclusion of boiled raisins in sandfly pots by the Indian workers and the addition of saline to the infecting material by Adler and Ber (1941) which was thought to have resulted in laboratory transmission. For this to be achieved it is also necessary to keep flies alive after oviposition, a problem which has foiled many of those who have worked on laboratory sandflies.

The actual mechanism of transmission is still somewhat in doubt, although as mentioned above, the empirical use of raisins or salt clearly produced some fundamental change in flagellate distribution which did not occur when these substances were not available. Napier (1946) suggested that transmission of *Leishmania* may be similar to that of *Yersinia pestis* by the flea, the parasites blocking the foregut so that no blood could pass through during the act of biting, resulting in regurgitation and ejection of the parasites into the wound. However, Adler and Theodor (1957) have rejected this theory on the grounds that the buccal cavity and pharynx have powerful dilator muscles and the epithelium could expand to allow blood to pass through into the stomach. They suggested that flagellates were simply deposited into the wound if the distal part of the proboscis was infected (as described by Wenyon, 1932). They believed no flagellate activity was necessary at deposition as parasites could be recovered from a Hertig apparatus or from skin after the bite of an infected fly. However, the Napier theory should be re-examined, for although the buccal cavity and pharynx may have dilator muscles a blocked oesophagus or oesophageal valve may not be capable of allowing parasites through initially. Evidence for this has come from studies by Strangways-Dixon and Lainson (1962, 1966) and Williams (1966). Working with *Le. m. mexicana* in *Lu. pessoana* and *cruciata* respectively, they found that transmission occurred when the sandflies probed the skin and failed to ingest blood $3\frac{1}{2}$–4 days after the infective feed, suggesting that infections occurred when promastigotes had interfered in some way with the feeding process. At this time, it is unlikely that heavy pharynx infections are found but oesophageal valve infections suggest that a blocked oesophageal valve may be important in transmission. Williams (1966) also found that a fly which had transmitted an infection no longer had flagellates in its alimentary tract. The functional morphology of sandfly mouthparts has recently been described by Lewis (1975). It is clear that the mechanism or indeed mechanisms of transmission by bite and the factors influencing the deposition of flagellates in the wound during the bite need to be much more thoroughly studied.

3. *Vector specificity in Old World strains*

Sinton (1925) suggested that on the Indian subcontinent *Ph. sergenti* and *Ph. argentipes* were the vectors of cutaneous and visceral leishmaniasis

respectively, as the distribution of these sandflies coincided with the distribution of the two conditions. This suggestion was one of the first to indicate vector specificity even before the details of the life-cycle and transmission were known. The subsequent work by Adler and co-workers in the Mediterranean Basin indicated the extent of this specificity even if the nature of it remains undetermined. Lewis (1974) tabulates those species of sandflies which have been incriminated as vectors of leishmaniasis in the various foci of the disease.

The studies on the behaviour of *Leishmania* in sandflies in China have been reviewed by Chung (1953). He cites the work of Young and Hertig (1926) and Patton and Hindle (1927), who described the development of *Le. donovani* in *Ph. chinensis*. Patton and Hindle (1927) observed that the flagellates in *Ph. chinensis* attached themselves to the midgut lining and grew forward and invaded the pharynx and buccal cavity in 6 days. If the fly became infected it would remain so throughout its life. However, *Le. donovani* behaved in quite a different manner in *Ph. sergenti*. Initial development occurred in the midgut but the parasites died out in 3–4 days. Development differed from that in *Ph. chinensis* as the flagellates did not attach to the midgut wall or invade the anterior part of the gut, the infection being restricted to the posterior broad part of the midgut. No invasion of the oesophagus was observed in 430 flies which were fed on infective material. This study represents an early example of the view that attachment is an important criterion in the assessment of the vector potential of an insect host and that for an infection to develop its full potentiality attachment must occur. Later observations by Feng (1951) indicate that *Ph. mongolensis* is a poor vector of *Le. donovani* (see Section IV).

Adler and Theodor (1929) were able to infect *Ph. sergenti* with *Le. tropica* from human oriental sores and obtained much higher infection rates in this fly than with *Ph. papatasi* fed on the same lesion; they also obtained proboscis infections more readily in *Ph. sergenti*. This work suggested a difference in susceptibility of species of sandflies to leishmanias and this was confirmed in other situations. Adler and Theodor (1930) fed both wild and laboratory bred sandflies of the species *Ph. perniciosus* and *Ph. papatasi* on a Chinese hamster infected with visceral leishmaniasis. Fifteen out of eighteen *Ph. perniciosus* became infected but only a single fly out of 123 *Ph. papatasi*. Other figures for *Ph. major* and *Ph. sergenti* indicated that the former fly was a good vector and the latter a poor one. Figures similar to the above were obtained by Parrot *et al.* (1930) for *Ph. perniciosus* fed on a dog infected with *Le. infantum*. Further results were obtained by Adler and Theodor (1931) confirming that *Ph. perniciosus* is a better vector of *Le. infantum* than *Ph. papatasi* despite the fact that *Ph. papatasi* takes a larger bloodmeal.

Adler and Theodor (1932, 1935) found that the infection rate of *Ph. perniciosus* fed on dogs with *Le. infantum* was proportional to the number of parasites in the skin. In heavily infected dogs up to 90% of the flies may be infected, but the susceptibility of *Ph. perniciosus* to this *Leishmania* is such that 20% of flies will become infected when the parasites are not detectable by histological techniques. Similar heavy infection rates in *Ph. ariasi* fed on dogs infected with *Le. infantum* have been obtained (Rioux *et al.*, 1972).

Although from this work it is clear that some species of sandfly are more suited than others to developing *Leishmania* infections, it must be pointed out that there is geographical variation in the ability of *Leishmania* species to infect the same species of sandfly. Adler *et al.* (1938) showed that strains of *Le. tropica* from Crete produced low infection rates in *Ph. papatasi* compared with Jericho strains of *Le. tropica*, and this result was obtained with insects from both Crete and Jerusalem.

In Sudan, where *Ph. langeroni orientalis* is the vector of kala-azar, Davis (1967) suggested that anatomical differences may be significant in determining the ability of sandflies to develop and transmit leishmaniasis. He noted that the anterior midgut of *Ph. l. orientalis* is more voluminous and the cardia less clearly differentiated than in *Ph. papatasi*. He suggested that these gross anatomical differences between vectors of cutaneous and visceral leishmaniasis may be reflected in other areas.

The specificity of sandflies to *Leishmania* is a quantitative one as the resistance can be broken down by experimental feeding on large numbers of flagellates from either mammalian hosts or from cultures. For example, Adler (1947) fed 140 *Ph. papatasi* on amastigotes of a Sudan strain of *Le. donovani*; 130 of these flies became infected although it is not thought that this fly is a vector under natural conditions, as only 2 out of 51 *Ph. papatasi* became infected when fed on a hamster with the same strain.

Adler and Theodor (1957), however, remark that there is little or no resistance to infection in natural vectors but in the non-vectors considerable resistance occurs which can only be broken down by heavy doses of parasites. However, if this resistance is broken down the infection rate decreases with time and flagellates are destroyed in the stomach of the fly, though this is also influenced by the numbers in the infecting feed. Adler (1947) found that with the *Le. donovani* from the Sudan in *Ph. papatasi*, parasites disappear around 15 days.

A further factor which influences the infection rate in vector flies is the amount of serum in the infecting feed. Adler (1938) obtained high infection rates in *Ph. papatasi* with a Cretan *Le. tropica* by reducing the amount of serum in the feed to 10% (15 out of 16 flies), whereas with 50% of serum only 6 out of 51 flies became infected; on the other hand, a Palestinian *Le. tropica* with 50% serum infected 13 out of 13 sandflies. The Palestinian strain is transmitted in nature by *Ph. papatasi* whereas the Cretan one is transmitted by *Ph. sergenti*. A further factor which may influence specificity is the process of digestion, for Adler *et al.* (1938) found that there was no anterior migration of parasites when haemoglobin crystals were present whereas flies which had digested the bloodmeal normally develop a normal anterior infection of the oesophageal valve and beyond. The variations observed in behaviour of the strain of *Leishmania* in sandflies suggests that the pattern of infection in flies is determined by genetic factors in both fly and parasite.

Adler (1962) and Gunders and Yaari (1972) found that promastigotes carry a negative charge, and suggested that the direction of their movement was dependent on local charge changes in their vicinity mediated through pH and their effect on the pH of the bloodmeal, and feeds from plant juices; it is these factors which have an important bearing on flagellate distribution in the fly.

This has been particularly demonstrated by the remarkable success obtained in transmission experiments by the Indian workers (Shortt *et al.*, 1931; Smith *et al.*, 1940, 1941; Swaminath *et al.*, 1942) which had previously been so unsuccessful, when boiled raisins were given to fed flies; similarly the use of salt three times more concentrated than in blood by Adler and Ber (1941) resulted in the transmission of *Le. tropica* by *Ph. papatasi*. Both salt and raisins provided some crucial factor which was previously lacking and both were purely empirical measures which turned failure into success. Both measures must have had a fundamental effect on the distribution and behaviour of the flagellates in the foregut, for many sandflies had fed on both animals and man both in India and Israel, and failed to transmit leishmaniasis, but were on dissection shown to have massive foregut infections. An explanation of the two results has been suggested by Davis (1967), who proposed that sandflies may eliminate their crop contents as well as defaecate whilst feeding in order to provide a larger volume for the bloodmeal. Unloading might occur through the proboscis before blood is sucked, resulting in flagellates in the foregut being washed into the wound either mechanically or by a change in osmolarity which may release attached parasites. This hypothesis needs to be tested experimentally. Ashford (pers. comm.) believes that parasites could be flushed out by the forcing forward of the air pockets which form in the midgut after a bloodmeal is digested, in a similar manner to an air bubble forcing material along under a cover slip.

4. *New World strains*

Coutinho (1941) reported forms of "*Le. braziliensis*" in the pharynx of *Lu. pessoai*. Later work on various species of *Leishmania* in Brazilian sandflies was carried out by Coelho (1962, 1964, 1966) and Coelho *et al.* (1967a-e); Coelho (1966) obtained transmission of *Le. mexicana* by *Lu. longipalpis* and *Lu. renei* and studied the life-cycles of "*Le braziliensis*", *Le. mexicana*, *Le. donovani* and *Le. tropica* in these flies. Coelho (1966) believed that there is an absence of host specificity in the *Leishmania*–sandfly relationship in the New World and suggested that Old World species of *Leishmania* could be easily established in New World flies (e.g. *Le. tropica*, see Coelho *et al.*, 1967b), a suggestion also borne out by recent biochemical information on the visceral strains (Chance *et al.*, 1974; Gardener *et al.*, 1974). Coelho *et al.* (1967b,c) also illustrate short rounded forms of *Leishmania* in the fly and these may correspond to the opisthomastigote types observed by Molyneux *et al.* (1975b).

Hertig and McConnell (1963) fed five species of Panamanian *Lutzomyia* artificially on *Le. braziliensis* from NNN by Hertig apparatus (Hertig and Hertig, 1927; Hertig and McConnell, 1963). The overall infection rate found by this method of feeding was 81%. Compared with infections of Old World leishmanias which develop anteriorly in sandflies, the infections of *Le. braziliensis* in *Lutzomyia* produced rosettes adhering to the epithelium of the hindgut triangle; by the 4–5th day this part of the gut posterior to the midgut was covered with attached short broad parasites. Occasionally the hindgut triangle was distended with flagellates. By 3 days, however, infections of the more anterior midgut and cardia began to appear with concentration around

the oesophageal valve, which became packed with parasites. In some flies, growth in the oesophagus and pharynx was observed. Hertig and McConnell (1963) reported that infections of the cardia were a prerequisite for infections further forward.

Johnson *et al.* (1963) found that hindgut infections similar to those obtained experimentally occurred in naturally infected Panamanian *Lutzomyia;* thus in this area *Leishmania* strains behaved differently from the classic pattern of development. Over 5000 female *Lutzomyia* were examined; in 416 flies promastigotes were found in the hindgut, as well as in the midgut, rarely in the cardia. Only twice were parasites found in oesophagus or pharynx but about 20% of all infected flies had parasites in the Malpighian tubules. The lesions produced in hamsters by these sandfly infections are indistinguishable from those produced by human isolates in hamsters. The growth pattern of human strains in sandflies fed on hamster lesions is similar to that observed in naturally infected flies and also to the wild caught sandfly strains which infected hamsters.

Johnson and Hertig (1970) followed up this work further by showing that all *Le. braziliensis* isolates (from mammals, man and sandflies) behaved in a similar way in two species of *Lutzomyia*, *Lu. sanguinaria* and *Lu. gomezi.* All infected flies developed infections in the hindgut triangle with or without midgut infection. A Peruvian espundia isolate behaved in the same way. However, the same species of flies when fed on two strains of *Le. mexicana* developed only midgut infections and not even free flagellates were observed in the hindgut. Johnson and Hertig (1970) suggested that this characteristic of *Le. braziliensis* to develop in the hindgut triangle, with *Le. mexicana* only developing in the midgut, could be used as a taxonomic criterion. Lainson *et al.* (1973), working in Para State, Brazil, dissected large numbers of sandflies of the genus *Psychodopygus* and found 3 *Ps. wellcomei* out of 1656 dissected infected, 2 out of 175 *Ps. paraensis* and 1 out of 127 *Ps. amazonensis.* Infections in these flies were variable in pattern but some flies had small rounded parasites which were attached to the hindgut triangle; infections also were observed in the midgut, oesophageal valve and part of the oesophageal diverticulum. Rounded forms were also found in the foregut. Inoculation of these natural infections produced infections in hamsters characteristic of *Le. b. braziliensis.* The observations of the Panamanian workers and those in Brazil (Lainson and Shaw, 1972b; Johnson *et al.*, 1963) seem to suggest that the *Le. braziliensis* complex have hindgut infections in *Lutzomyia* and *Psychodopygus*, whereas *Le. mexicana* sspp. and Old World mammalian leishmanias develop only in the anterior parts of the gut. Attachment of *Le. b. braziliensis* to the hindgut of sandflies is by hemidesmosomes (Killick-Kendrick *et al.*, 1976).

The findings of Central and South American workers seem to indicate that the *Le. mexicana* complex of organisms do not display such a rigid host specificity as Old World *Leishmania* in sandflies (Williams, 1966; Coelho *et al.*, 1967a–e; Disney, 1968), parasites being capable of developing mature infections in several *Lutzomyia* species. However, the ecological isolation of several of these species with respect to host preferences, biting times and flight activity

are the main factors which influence infection rates rather than intrinsic susceptibility.

5. *Role of the peritrophic membrane*

Feng (1951) has reviewed the behaviour of Chinese strains of *Le. donovani* in two species of sandfly, *Ph. chinensis* and *Ph. mongolensis*. The development of the parasite differs markedly in these flies and Feng (1951) relates this to the differences in the structure of the peritrophic membrane. Reviews of the structure and significance of the role of the peritrophic membrane in other Diptera have been published by Stohler (1961) and Le Berre (1967). *Ph. mongolensis* is not regarded as a vector of *Le. donovani* whereas *Ph. chinensis* is. In *Ph. chinensis*, *Le. donovani* taken in via the bloodmeal are confined in a complete sac of peritrophic membrane, but around 48 h this sac breaks posteriorly and later fragments, releasing promastigotes into the midgut. Migration forward then occurs with cardia, oesophageal valve, pharynx, buccal cavity and mouthparts later becoming involved. The peritrophic membrane of *Ph. mongolensis* behaves differently; a complete sac is formed around the bloodmeal and if flagellates are present in the bloodmeal they decrease in numbers as the sac becomes smaller but does not break. The small sac passes into the hindgut and it is finally voided as faeces and the fly is freed of infection. The other species of sandfly studied by Feng (1951) was *Sergentomyia squamirostris*, a vector of *T. bocagei* (see Section IIB). The peritrophic membrane of this species forms a tightly closed sac around the bloodmeal but the posterior end is not tightly closed, and 24–48 h after feeding the blood is discharged into the hindgut. The flagellates are passed down this part of the gut and attach to the walls where further development occurs. Feng (1951) reported that the peritrophic membrane itself was a definite chitinous structure resisting the action of potassium hydroxide. Adler and Theodor (1926) described the peritrophic membrane of *Ph. papatasi*, *Ph. minutus* and *Ph. perniciosus* as a whitish amorphous structure which extended anteriorly as far as the cardia and posteriorly into the hindgut and was sealed at both ends. The peritrophic membrane of sandflies is itself a secretion type (Le Berre, 1967). Recent studies on the ultrastructure of the peritrophic membrane have been made by Gemetchu (1974) in *Ph. longipes* and by Killick-Kendrick *et al.* (1974) in *Lu. longipalpis* in relation to the development of *Le. mexicana amazonensis*. In *Ph. longipes*, the vector of *Le. aethiopica* (Ashford *et al.*, 1973b), the peritrophic membrane formed a discrete envelope around the bloodmeal 48 h after a feed, then began to disintegrate on the third day—a situation similar to that described by Feng (1951) for the other good vector of *Leishmania*. Gemetchu (1974) suggested that the membrane may be a manifestation of "physico-chemical interaction" between enzyme and the bloodmeal. Killick-Kendrick *et al.* (1974) found that the so-called peritrophic membrane of *Lu. longipalpis* was secreted from between the lining microvilli of the midgut and surrounded the bloodmeal. Parasites were found embedded in this membrane at an early stage of the infection and as the membrane broke up the parasites escaped to colonise other parts of the gut. However, the membrane remained associated with degenerated sloughed off cells liberated

into the lumen of the midgut between 2 and 3 days after the bloodmeal was taken, and parasites were still associated with the structure at this stage although many were free in the gut lumen also. The peritrophic membrane is perhaps a misnomer in sandflies, because these ultrastructural studies indicate that it is a diffuse secretion with little structural definition. It is clear, however, that this "membrane" performs an important role in the determination of vector specificity in sandflies and perhaps a role in the development of the parasite in the fly, protecting the parasite from immediate direct confrontation with secretions prior to transformation and thus adaptation.

6. *Effects of* Leishmania *on sandflies*

Ashford *et al.* (1973b) found that *Le. aethiopica* in *Ph. longipes* may exhibit a degree of pathogenicity in the fly. This observation was based on dissection in the field of *Ph. longipes* which had obtained their infection from hyraxes. Flies which were caught in the field and had fresh blood had an infection rate of 6%. Flies with old blood, old blood and developed eggs or just with developed eggs had infections rates of 8·8%, 11·7% and 11·5% respectively. A lower rate of 4·8% was observed in parous female *Ph. longipes* in the same area. One possible explanation for this 50% drop in infected flies between these two groups is that the parasite was causing a degree of mortality. Saf'janova and Alexseev (1975; see also Section VIC) reported that the presence of two species of *Leishmania*, *Le. tropica* and *Le. gymnodactyli*, in artificially fed *Ph. papatasi* was "harmful" to flies. These findings are of interest when compared with those of Watkins (1971a,b), Bailey and Brooks (1972a,b) and Smirnoff and Lipa (1970). These authors (see Section IIIA1a and 1b) reported the pathogenicity of various flagellates to insects associated with haemocoele invasion. The observations of Killick-Kendrick *et al.* (1974) and Molyneux *et al.* (1975a,b) are of interest in this respect. Killick-Kendrick *et al.* (1974) showed that flagella of nectomonads of *Le. m. amazonensis* penetrated the degenerating midgut epithelial cells of *Lu. longipalpis* which had shed their microvilli. These flagella appeared in tunnels separated from the cytoplasm of the cells by a host cell plasmalemma. However, the long nectomonad flagella were found closely associated with the *muscularis* layer lining the midgut by Molyneux *et al.* (1975a), who suggested that the haemocoele may become involved. In a later study (Molyneux *et al.*, 1975b) parasites themselves were found inside midgut cells, in some cases separated from the cytoplasm by a sandfly cell plasmalemma, though in other situations parasites were in direct contact with midgut cell organelles and several parasites were observed in a single host cell. Adler and Theodor (1929), however, found *Le. tropica* in the coelom and muscles of *Ph. sergenti*; they suggested that this was of no significance and was associated with unhealthy flies. The significance of these observations is not yet fully clear. It is possible that intracellular invasion of host midgut cells by *Leishmania* results in pathogenic effects similar to those documented by Garnham (1955) when *T.* (*H.*) *lewisi* invades the midgut cells of fleas (see Section IIIA1b). This observation would explain the results of Ashford *et al.* (1973b); it is suggested that in future work on sandflies in the field, workers should attempt to clarify and obtain more data relating to these observations.

## V. Monoxenous Trypanosomatids in Insects

The biology of this group of parasites has been reviewed by Lipa (1963) and Wallace (1966). The former worker discussed the pathology of the insect flagellates, and Wallace (1966) gave a valuable checklist, generic definitions, host records and geographical distribution of known arthropod flagellates (mainly insect) known to that date. Since these reviews, further work on the relationship between these parasites and their hosts has been completed and the main emphasis of this section will be on this aspect of the work.

### A. *Crithidia*

Brooker (1971c) has given a detailed description of the fine structure of *Crithidia fasciculata* in culture. Earlier, Brooker (1970) described the desmosomal mechanisms of attachment of the flagellum to the reservoir as it emerges and these types were referred to as Type A desmosomes, which were a permanent feature of the cytological organisation. Type B desmosomes and hemidesmosomes linked the flagellates together in rosettes. These junctions are believed to enable rosettes to maintain their integrity. Brooker (1970) thought that the Type B desmosomes and hemidesmosomes were very similar morphologically, the difference being that Type B desmosomes were formed between flagella whereas hemidesmosomes were found between flagella and inorganic material and between flagella and insect tissues. The desmosome and hemidesmosome represented different manifestations of the same "adhesive property". The conditions under which these junctions are formed by flagella have not been defined. Brooker (1970) demonstrated that rosettes of *H. muscarum* and *C. fasciculata* could be formed together in culture and that desmosomes between their flagella could be formed. This indicated that junctional attachments were non-specific although the flagellum of one parasite was never seen to adhere to the body of another.

Brooker (1970) quotes Ross (1906) on the factors which may affect flagellar adhesion. Ross observed that when *Crithidia* haptomonads in mosquito larvae came into contact with water, they displayed active movement and broke away from the gut wall. It appeared that changes in tonicity could result in the breakdown of these junctions.

Later, Brooker (1971b) showed that flagella of *C. fasciculata* would adhere by hemidesmosomes to millipore filters and flagella were inserted between the pores of the filters. He showed that haptomonads detached if distilled water was added to the medium and they transformed to free swimming nectomonad forms. When *C. fasciculata* was examined in the mosquito *A. gambiae* (Brooker, 1971a) the parasites were observed to adhere to the cuticular surfaces of the hindgut. The hemidesmosomal junctions were the same in all regions of the gut examined by Brooker (1971a). The tip of the attached flagellum was expanded and the adherent flagellar membrane followed the contour of the cuticular surface to which it was attached. In no case did *C. fasciculata* penetrate the cuticular lining of *A. gambiae*. The length of the flagellum of individual parasites varied depending on the distance of the parasite body from the gut

wall. When the parasites were close the flagella hardly emerged from the reservoir before they were attached. If an individual was separated from the epithelium by a layer of parasites the flagellum was longer, probing between them before it attached. Brooker (1971a) found that in the hindgut the parasites adhered in clumps around evaginations of the epithelium, giving a rosette appearance. When water was added to the gut detachment occurred within 5 min and the number of filaments emanating from the hemidesmosomal plaque was reduced. However, the band between the flagellar membrane increased in density and deep invaginations of the flagellar membrane appeared as pieces of membrane moved away from the gut wall. These changes occurred prior to flagellar lengthening, detachment and transformation to nectomonads.

A further morphogenetic change associated with attachment of *Crithidia fasciculata* to insect gut was described by Brooker (1972). It was observed that attached deformed flagellates, packed into folds of the rectum, had groups of subpellicular microtubules sunk below the plasmalemma, and this allowed a change of shape to occur which would not otherwise be possible. The change was possibly stimulated by the forces of the movement of the gut in the immediate vicinity of the parasites, for this arrangement was not seen in culture forms and was more common in heavily infected mosquitoes. This idea was confirmed when culture forms of *C. fasciculata* were forced through a 0·22 $\mu$m pore size millipore filter and similar microtubules were commonly found. Brooker suggested that this phenomenon was unlikely to be observed only in *C. fasciculata* and might occur in similar crowded situations in the guts of insects. Molyneux *et al.* (1975b) have observed similar sunken microtubules in promastigotes of *Le. m. amazonensis* in the sandfly midgut but these appeared to be associated with autolysis of the parasite.

Schmittner and McGhee (1970) have studied responses of three experimental insect hosts (*Drosophila viridis*, *Tenebrio molitor*, *Acheta domesticus*) to infections of six different *Crithidia* species, introduced by inoculation into the haemocoele. All *Crithidia* species inoculated were lethal to *D. viridis*. A phagocytic response was evoked and up to 30 flagellates were found in a single phagocytic cell. In *Tenebrio molitor*, *Crithidia fasciculata* was the most lethal. Phagocytic reaction to the flagellates was similar to that in *Drosophila*. Encapsulation of the flagellate by haemocyte aggregation occurred; brown-black masses of 60 $\mu$m–900 $\mu$m in diameter were formed which contained variable numbers of flagellates. Encapsulation was not total as flagellates could be washed free from the aggregates. *Acheta domesticus* was least susceptible to *Crithidia*, and death from the infection of this species was not observed. Large foreign-body nodules were formed in 60% of infected *A. domesticus* and the process was initiated rapidly, as nodules could be seen developing within 24 h. Most hosts only developed a single nodule. No tissue or organ penetration by *Crithidia* species was observed. These observations on haemocoelic encapsulation of flagellates would repay closer investigation, as clearly some parasites are capable of evoking a response whereas others are not, or the host is incapable of mounting this type of defence reaction to trypanosomatids, or it has not yet been observed; e.g. *R. prolixus* and *T.* (*H.*) *rangeli* (Watkins, 1971a,b), *Glossina* and *T.* (*T.*) *brucei* (Mshelbwala, 1972).

Ilan *et al.* (1969) and Injeyan and Meerovitch (1974) have recently found that insect juvenile hormone (JH) has an inhibitory effect on *Crithidia fasciculata* growth and they suggested that this is caused by interference with RNA synthesis at the level of the plasmalemma. The opposite effect to JH on *Crithidia* has been observed in the presence of various pteridine compounds which have a stimulating effect on *Crithidia*. Various aspects of the biology of these compounds are reviewed by Zeigler and Harmsen (1969). The marked effect on growth of *Crithidia* by these compounds and their variety and wide distribution in insects suggests that more work should be carried out on the biology of pteridines in vectors of trypanosomatids, in addition to investigating whether such compounds have growth-stimulating effects on species of *Trypanosoma* and also *Leishmania*. The distribution and localisation of such compounds may be the basis for vector parasite specificity, a general explanation of which has not been forthcoming. Further information on the structure and distribution of pteridines and other key references to this subject can be obtained from Zeigler and Harmsen (1969).

### B. *HERPETOMONAS*

The most recent work on this genus, not included in Lipa (1963) and Wallace (1966), is reviewed in this section.

Smirnoff and Lipa (1970) described a new species of *Herpetomonas*, *H. swainei*, a parasite of the sawfly *Neodiprion swainei* (Tenthredinidae: Hymenoptera). This insect is a serious pest of jackpine trees in Canada. Naturally infected sawfly larvae did not show any gross changes in external appearance in both experimentally and naturally infected insects, there was no loss of appetite, their movements were normal and so was their frass. The parasite overwintered in the cocooned insect; the numbers of emerging adults, their period of emergence and the fecundity of females was not affected by the infection of *H. swainei*.

When larvae were infected the flagellates lined the gut wall in 6–8 days and were found in the Malpighian tubules 12–16 days later and thereafter in the haemolymph. All larval instars were affected but the flagellates were most common in third and fourth stage larvae. Mortality was seen in fourth and fifth instar larvae but never exceeded 20% and was observed in individuals which had been infected early in life, suggesting that death was due to parasite numbers which resulted in invasion of haemolymph. Smirnoff and Lipa (1970) also reported a reduction in fatty tissues in infected larvae (second and third instar). Adult male sawflies were more heavily parasitised; this was suggested to be due to juvenile hormone levels. This suggestion is most interesting in view of findings of Ilan *et al.* (1969) and Injeyan and Meerovitch (1974) (see Section IVA). *H. swainei* has been used as a trial biological control agent (Smirnoff, 1974).

Bailey and Brooks (1972b) have investigated the effect of *Herpetomonas muscarum* on the eye gnat, *Hippelates pusio*. Culture flagellates were used to infect larval gnats, which were the most susceptible stage. The mortality in larval gnats depended also on the temperature at which the larvae were kept.

The highest mortality due to the flagellates was observed when the larval period was longest at 18·5°C, and although the higher temperatures were better for flagellate development (optimum 29·5°C), mortality was lower at these temperatures. Under standard rearing conditions (26·9°C, 16 h light, 8 h dark) mortality due to *H. muscarum* was 48 %. As in *H. swainei*, *H. muscarum* in *Hippelates* did not affect developmental period or gnat emergence. Only 6% pupal mortality was observed when the gnats were infected, and the maximum effect was at 29·5°C.

Histopathological studies of larvae (Bailey and Brooks, 1972a) showed that mortality was due to lesions in the larval gut which allowed bacteria in to infect the haemocoele. *H. muscarum* was initially found in the anterior midgut spreading to the gastric caeca and posterior midgut. The flagellates probably penetrated the gut in the gastric caeca or anterior midgut and set up haemocoelic infections which later resulted in bacterial "septicaemia". Phagocytosis of flagellates was infrequent and no encapsulation of parasites was observed. The bacterial infection of the haemocoele resulted in death of the flagellates and killed the larvae. Electron micrographs of the gut showed *H. muscarum* between clefts in the epithelial cells of midgut and gastric caeca and closely associated with the microvilli of the epithelium; the peritrophic membrane of larval *H. pusio* was highly folded and parasites were observed in the ecto-peritrophic space, whereas in the posterior midgut the flagellates were within the endoperitrophic space. Other electron micrographs of Bailey and Brooks (1972a) revealed *H. muscarum* inside intact and apparently healthy anterior midgut epithelial cells of *H. pusio* larvae. These parasites were in direct contact with the cytoplasm, separated only by their own plasmalemma. As the haemocoele of the eye gnat became more heavily infected with bacteria, the midgut epithelium degenerated, and about 48 h after the flagellates first invaded, the bacteria overgrew the flagellates numerically and little epithelium remained. Bacterial infections clearly were the cause of death in this system, but Bailey and Brooks (1972a) believed that the flagellates themselves were capable of serious pathological effects although no other tissues were invaded except the gut wall. If the larvae could be reared under axenic conditions this problem could be investigated, but this was not possible. The mechanism of flagellate invasion of the haemocoele in this infection is not known, but Bailey and Brooks speculated that it may be by continuous mechanical action, although it will be recalled that Watkins (1971a) suggested that *T.* (*H.*) *rangeli* secreted enzymes which altered the basal lamellae of the gut cells of *R. prolixus*. Further studies on the effects of *Herpetomonas* infections in insects may aid the study of digenetic pathogenic trypanosomatids whose vectors are less easy to study.

Brun (1974) has reported the method of attachment of forms of "*H. mirabilis*", a variant of *H. muscarum*, in the hindgut of *Chrysomyia chloropyga*. The whole surface of the hindgut wall was covered with a layer of flagellates and hemidesmosomes were formed on the inner layer of the flagellar plasmalemma (Figs 14, 15). There was a great enlargement of the intraflagellar area and pinocytotic intuckings of this flagellar membrane were observed (Figs 14, 15). Adjacent flagella from different individual parasites were attached

by flagellar desmosomes, although no attachment between parasite bodies or parasite body and flagella were seen. Flagella of "*H. mirabilis*" in *C. chloropyga* belonging to those flagellates not directly in contact with the cuticle of the hindgut interdigitated between the initial flagellate layer and attached by the same method as those closest to the wall of the gut. Brun (1974) did not observe any cystic stages in his study of the flagellate in the fly. In the midgut of the fly parasites were found in the endo- and ectoperitrophic spaces. In the former region they were packed in such a way as to obliterate the lumen of this space and were packed tightly against the inner wall of the tube formed by the peritrophic membrane (Fig. 13).

### C. OTHER GENERA

Lipa (1963) and Wallace (1966) have reviewed the monogenetic flagellate genera *Leptomonas*, *Blastocrithidia* and *Phytomonas*. Loŭcková (1974) reported the attachment of a *Leptomonas* sp. in the midgut of *Corixa punctata*. The parasites formed a carpet-like layer and rosettes were present in the lumen. The attached parasites were promastigotes with a short flagellum. Cyst formation was observed but the detailed morphogenesis of this process has not yet been reported. Molyneux and Ashford (1975) have observed attachment of a flagellate in the hindgut of a flea, *Peromyscopyslla silvatica spectabilis*. In this site a heavy infection of an unknown flagellate lined the epithelium. The flagella of the parasites had expanded intraflagellar areas; a desmosome-like junction occurred between flagellar membrane and cuticle and the cuticular folds of the hindgut were occupied by expanded flagella. Lipa (1963) reported that when some parasites (*Leptomonas*) were observed by the light microscope attached to the flea hindgut, the forms were short and stumpy. This was a feature of the parasite studied by Molyneux and Ashford (1975). The reservoir of the flagellate in the flea was obliterated and it was impossible to decide to which genus it could properly be assigned. *Leptomonas* reputedly is passed out as an encysted amastigote in the dejecta and can then infect larvae. Although they saw no parasites which could be identified as a cyst, Molyneux and Ashford (1975) observed parasites with subpellicular microtubules made up into doublets and triplets. This does not seem to have been reported elsewhere in trypanosomatids and may be associated in some way with cyst formation in the insect.

## VI. EFFECT OF OTHER ORGANISMS ON TRYPANOSOMATIDAE IN INSECTS

### A. BACTERIA: SYMBIOTE–TRYPANOSOMATID INTERACTION

The presence of symbiotes in insects has been recognised for many years and a wide variety of cells and insects are infected (Lanham, 1968). In addition to these essential symbiotes other bacteria may be present which on invasion of certain tissues may be pathogenic. The presence of flagellates may evoke damage which results in such pathogenic effects.

The best defined of these symbiotes associated with trypanosomatid vectors

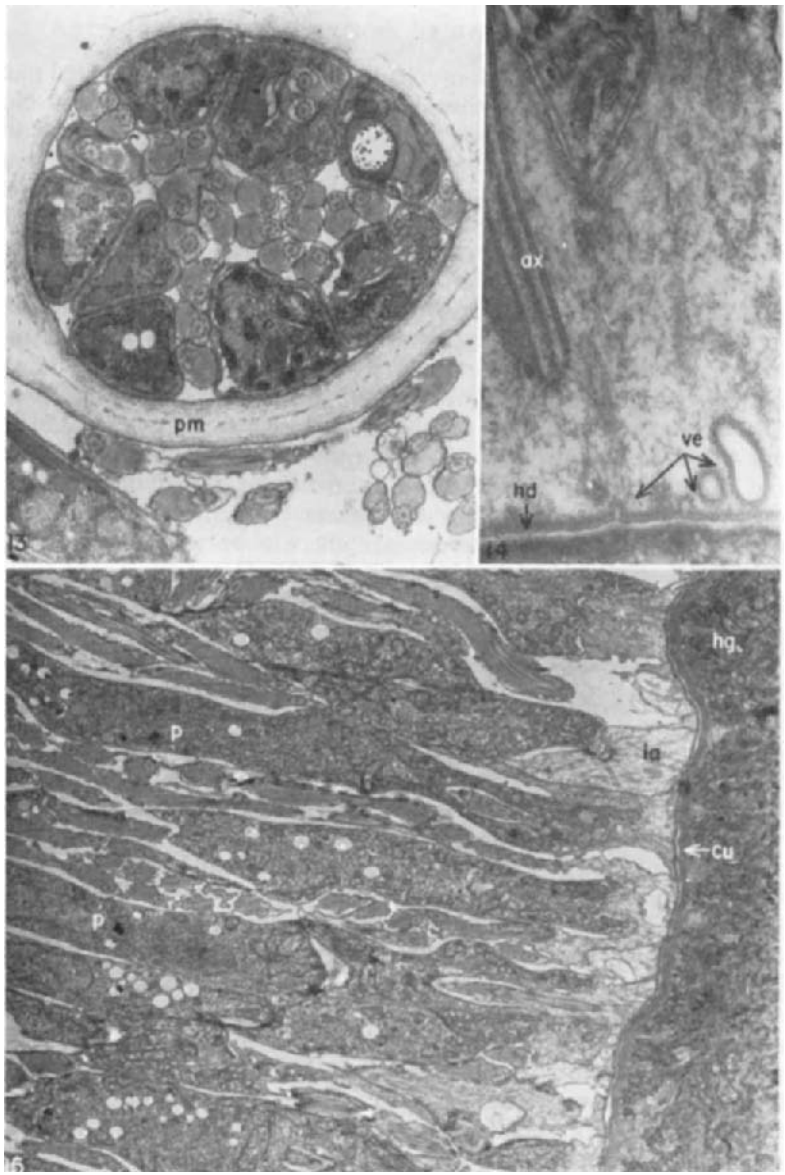


FIG. 13. *Herpetomonas muscarum* packed into endoperitrophic space of *Chrysomyia chloropyga* midgut. Parasites are also present in ectoperitrophic space. (Peritrophic membrane, **pm**.) × 14 300.

FIG. 14. Attachment region of "*H. mirabilis*" in ectoperitrophic space of hindgut of *C. chloropyga*. Hemidesmosome (**hd**, arrowed) is method of attachment, and pinocytotic vesicles are observed (**ve**, arrowed) through which material is possibly taken up from the epithelium. (Axoneme, **ax**.) × 50 000.

FIG. 15. Lower power micrograph of "*H. mirabilis*" in same site as Fig. 14, showing pile carpet effect of parasites (**p**). (Intraflagellar area, **ia**; hindgut, **hg**; cuticle, **cu**, arrowed.) × 6200.

(Figures 13, 14 and 15 from Brun, 1974; reproduced by kind permission of the author and of the Editors of *Acta trop.*)

is *Nocardia rhodnii* found in *R. prolixus*. These organisms are found in cells lining the anterior segment of the midgut and they are liberated into the blood-meal when the epithelium is stretched in feeding. *N. rhodnii* provides vitamins for the bug (Lake and Friend, 1968) (see Section IIIA1a). The relationship between *N. rhodnii* and *T. cruzi* was studied by Mühlpfordt (1959), who found no effect of *N. rhodnii* on the course of infection of *T.* (*S.*) *cruzi* in the bug. *N. rhodnii* does not provide any essential factor for transformation of trypomastigote to epimastigote, as this happens in bugs which are sterile as well as in symbiote-free bugs. Brener (1973), in a review of *T.* (*S.*) *cruzi* biology, states that no correlation has yet been found between *T.* (*S.*) *cruzi* development in the bug and the intestinal flora.

Cavanaugh and Marsden (1969) isolated various bacteria from *R. prolixus* and *T. infestans* and concluded that bacterial flora may be important in determining bug susceptibility to *T.* (*S.*) *cruzi*; they suggested that further studies should be planned. Jadin (1967) reported favourable growth of a *T.* (*S.*) *cruzi* isolate in culture in the presence of a *Pseudomonas* but it seems clear from the above results that there is no conclusive effect of bacteria or symbiotes on *T.* (*S.*) *cruzi* in reduviids.

The effects of *T.* (*H.*) *rangeli* on *N. rhodnii* have been discussed earlier (see Section IIIA1a).

There are many reports of symbiotes in *Glossina* species but the effects of such infections on tsetses and their trypanosome infections are unknown (Reinhardt *et al.*, 1972; Wei-Chun Ma and Denlinger, 1974; Pinnock and Hess, 1974; Huebner and Davey, 1974). Besides these studies Jadin *et al.* (1966) reported the isolation of 23 strains of *Pseudomonas* from 289 *G. f. quanzensis* in Kinshasa, Zaire, but no trypanosome associations were found. Burtt (1946b), however, observed that a significantly higher proportion of trypanosome-infected flies had bacteria than uninfected ones. These bacteria, however, did not affect the infectivity of the trypanosomes. Lloyd (1930) and Peel (1962) reported that trypanosomes in the gut of *Glossina* can be destroyed by bacteria, a view supported by Jordan (1964) and Baldry (1969). Riordan (1971) also suggested that *T.* (*T.*) *brucei* midgut infections could be destroyed by bacteria, explaining the finding of salivary gland positive flies which had negative midguts. Jadin (1967) has reviewed this topic but, as with *T.* (*S.*) *cruzi*, nothing definitive can be stated with regard to these relationships.

The effect of bacteria on *Leishmania* infections has been discussed by Adler and Theodor (1957) (see Section IVB). Molyneux (1976) discussed the relationship of *Herpetosoma* trypanosomes and bacteria in fleas.

### B. VIRUS–TRYPANOSOMATID INTERACTION

The only quantitative study on virus–trypanosome interaction was by Beinz (1968), who studied the interaction between ornithosis virus and *T.* (*T.*) *b. brucei* at 25°C in cultures of FL cells and found that growth was inhibited. However, at 37°C in culture, no influence could be detected. The inhibition of *T.* (*T.*) *brucei* at 25°C in the presence of virus is of considerable interest in view of the recent findings of Jenni (1973) and Jenni and Steiger (1974).

Jenni (1973) reported finding virus-like particles (VLPs) in cytoplasmic vesicles in the salivary glands of *G. morsitans centralis*, and Jenni and Steiger (1974) have found VLPs in the nucleolar region of midgut muscle of cells of *G. f. fuscipes*. The relationship between both these types of particle to *Glossina* itself and to trypanosome infections in flies in particular is not yet known.

The VLPs described by Jenni (1973) from salivary glands and their association with rods resemble those described by Molyneux (1974) in the cytoplasm of the *Leishmania* parasite, *Le. hertigi*, a parasite found normally in the skin of the tropical porcupine *Coendou rothschildi* in Panama (Fig. 16). These VLPs were also associated with cytoplasmic rods of two types (Molyneux, 1974; Molyneux *et al.*, 1975a). There was no evidence that the particles caused any reduction in the ability of the isolate to grow in culture. It is possible that the VLPs of *Le. hertigi* were derived from a sandfly infected with a virus similar to that described by Jenni (1973). Virus–trypanosomatid interaction in vectors should be studied in order to define the importance of concurrent infections.

Smirnoff and Lipa (1970) described a new flagellate parasite of *Neodiprion swainei*, *Herpetomonas swainei*, and during their studies a virus of *N. swainei* was also found. This polyhedral virus caused some mortality in the insect but it was stated that viral development was not affected by the flagellate and that both organisms developed independently in the same host.

### C. INTERACTION BETWEEN TRYPANOSOMATID SPECIES

Under natural conditions there are several situations in which an individual vector may be infected with more than one species of trypanosomatid. Thus, the effects of one parasite on another may be important in the context of competition for sites of establishment and available vector resources or because one species may be pathogenic to the vector, thus reducing the transmissibility of both parasites.

Mixed infections between trypanosomes and monoxenous flagellates will occur in many situations but few have been well documented. The recent work of Cerisola *et al.* (1971), however, emphasises the importance of such studies. These authors described *Blastocrithidia triatomae* from *Triatoma infestans* in Argentina. The finding of this parasite renders it essential for workers on Chagas' disease to maintain constant surveillance of laboratory colonies used in xenodiagnosis for this parasite, and for field workers recording infection rates to be able to identify *Blastocrithidia triatomae*. The important feature of this organism is the bacillus-like corpuscles or cysts found attached to the flagellum. These forms are also called straphangers (McGhee and Hanson, 1962). Cerisola *et al.* (1971) described no effects of *B. triatomae* on *T. infestans* or on the biology of mixed *T. (S.) cruzi–B. triatomae* infections.

Flagellate parasites have been reported from many insects (Wallace, 1966) and some of these hosts are known to be vectors of trypanosomes. These organisms may affect the behaviour of trypanosomes in vectors but quantitative analysis of any such affects have never been estimated.

Saf'janova and Alexseev (1975) used an artificial feeding technique and were able to infect *Phlebotomus papatasi* and *Sergentomyia arpaklensis* with two

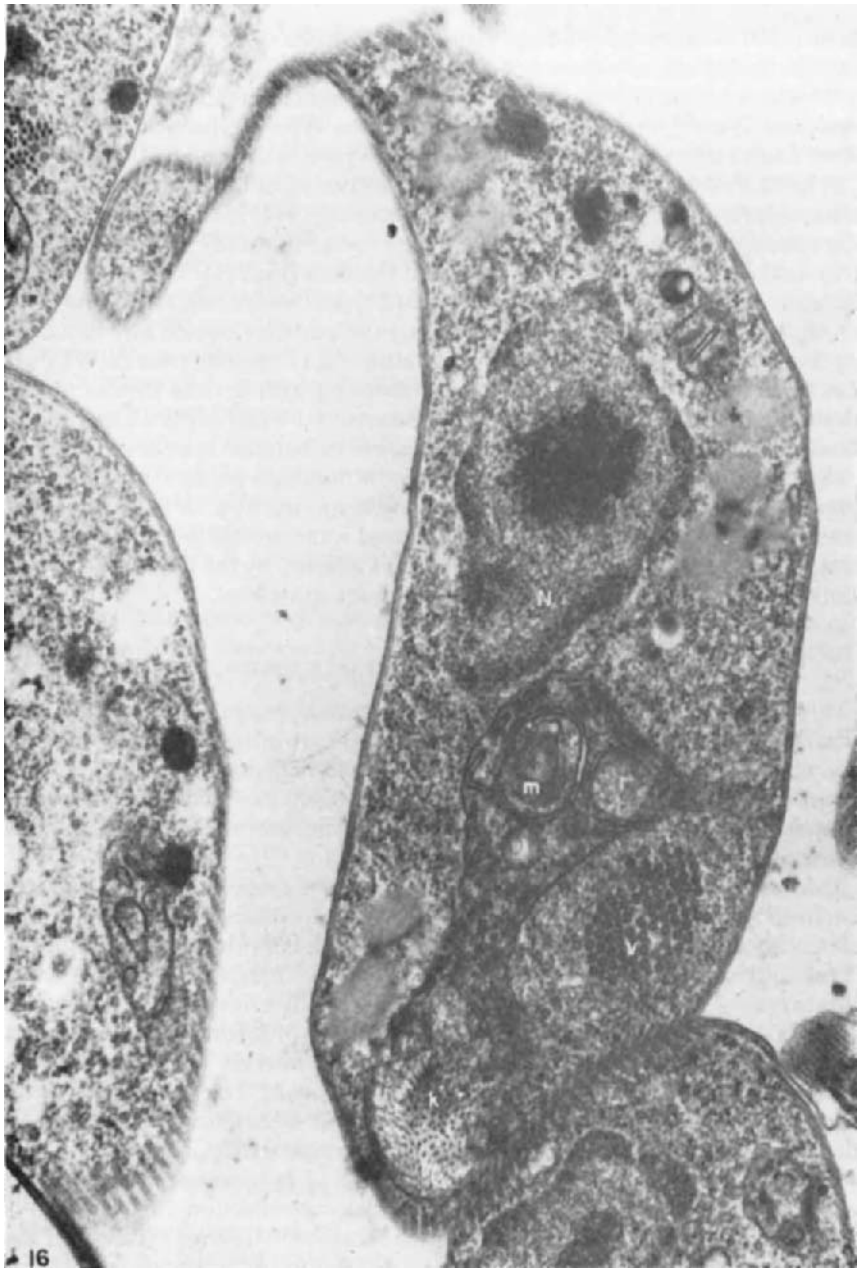


FIG. 16. Longitudinal section through cultured promastigote of *Leishmania hertigi*, showing group of virus-like particles (**v**) and inclusions (**i**) stimulated by the presence of such particles in another parasite. Nucleus, **N**; mitochrondrion, **m**; kinetoplast, **k**. × 30 000.
(From Molyneux, 1974; reproduced by kind permission of *Nature*.)

*Leishmania* species, *Le. tropica major* and *Le. gymnodactyli*. The longevity of the sandflies varied depending on the strain of *Leishmania* with which they were infected. *Ph. papatasii* survived longer if infected with *Le. t. major* compared with the reptile strain, and *S. arpaklensis* survived less time with the *Le. t. major* than with the *Leishmania* of the lizard. Mixed infections in sandflies were obtained. The possibility that sandflies could have mixed *Leishmania* in nature was shown by serological testing of parasites taken from the experimentally infected flies. The finding that there was reduced longevity in the flies infected with strains which cannot be regarded as homologous reinforces the view that *Leishmania* infections may be pathogenic to sandflies (Ashford *et al.*, 1973b; Molyneux *et al.*, 1975a).

Mixed infections of *T.* (*S.*) *cruzi* and *T.* (*H.*) *rangeli* are commonly found in nature, particularly in *Rhodnius prolixus*. D'Alessandro and Mandel (1969) reported that the presence of *T.* (*S.*) *cruzi* in the intestine did not prevent *T.* (*H.*) *rangeli* from establishing an infection in the bugs. However, if a bug infected with *T.* (*S.*) *cruzi* became infected with *T.* (*H.*) *rangeli* and pathogenicity resulted this would be of importance in the epidemiology of Chagas' disease (Marinkelle, 1968a).

Although under field and experimental conditions *Glossina* is capable of developing of established infections of four subgenera of mammalian trypanosomes (*Trypanozoon*, *Duttonella*, *Nannomonas*, *Pycnomonas*) and one reptilian species, *T. grayi*, most flies if infected at all harbour a single species, rarely two. No information is available regarding the relationship between the infections in *Glossina*, or the effect of an established infection of one species on the ability of another to establish.

### D. OTHER MICRO-ORGANISMS IN VECTORS AND TRYPANOSOMATIDS

Little work on the relationship between yeasts or fungi and trypanosomatids is available but the importance of such relationships is evident from the findings of Lehmann (1970), who reported that *T.* (*S.*) *cruzi* cultures grown in association with *Candida* species had more enzymes than control uncontaminated cultures. The enzyme pattern of formerly contaminated cultures was maintained after animal passage and Lehmann suggested that the character was heritable and transformation had occurred. Lehmann points out that no information is available on natural fungus flora of reduviid bugs. Changes in *T.* (*S.*) *cruzi* enzymes may occur in bugs infected with such a flora and such changes could be reflected in the parasite, which might have dramatic biological effects.

McConnell and Correa (1964) reported that a Panamanian sandfly, *Lutzomyia vespertilionis*, which is normally associated with bats had a high infection rate with a trypanosome. Other organisms besides trypanosomes were present in the haemocoele of sandflies of various species. 58% of females of *Lu. vespertilionis* with fungal hyphae in the haemocoele had flagellate infections, also indicating that the flagellate/fungal infections were not detrimental each to the other. Nematodes, probably of the genus *Tylenchinema*, or filariids were found in the haemocoele of these flies and some had concurrent flagellate infections. The presence of the nematode did not prevent establishment of the flagellate infection.

Rioux *et al.* (1972) have found a species of *Adelina* in *Sergentomyia minuta* and *Phlebotomus perniciosus*. Although observations could not be made on the effects of such infections on *Leishmania*, these authors list those protozoa which have been found in sandflies, the species of fly infected and references.

## VII. Conclusions

The modification of flagellar structure observed in trypanosomatids in their insect hosts has emphasised that flagella can function as adhesive as well as locomotory organelles. The most common feature of all these attached flagella is the condensation of material on the inner leaflet of the flagellar plasmalemma which has been termed a hemidesmosome, and which may or may not be accompanied by the presence of filaments within the flagellar sheath. This type of attachment is usually to stomodaeal and proctodaeal ectodermal parts of the gut which are lined with cuticle of various types, except in one example where such attached forms have been found in salivary gland epithelium which has a brush border and in this situation also no expansion of the intraflagellar region occurs (Steiger, 1973). Table VII tabulates the parasites, site of attachment and the structure of the epithelium of the hosts which have so far been examined.

It is clear that the ability of a parasite to transform in the insect and then to attach to midgut as well as to both endocuticle and epicuticle requires great adaptive capability. What is of interest in this work is the similarity observed in the type of attachment employed in a variety of parasite genera, though the biochemistry and biophysics of the method itself are not yet understood.

The review by Curtis (1972) provides a summary of the methods of cell adhesion which are observed in various systems, and indicates the possible mechanism which may apply in the systems discussed in this review. Vickerman (1972) supports the view that adhesion of protozoa to cell surfaces may be of the "secondary minimum" type, but the specificity of trypanosomatid attachments tends to support the bridging hypothesis (Curtis, 1972). In most insect–trypanosomatid systems that have been studied (see Table VII) where high infection rates in vectors are obtained, the sites of attachment are cuticle lined surfaces. The situation in the Salivaria section trypanosomes is most interesting as there seems to be a relationship between the infection rate in the vector and the site of development of the vector forms of the trypanosome. The highest rates are observed in the subgenus *Duttonella* where development and attachment only occur in the proboscis; *Nannomonas* comes next lying between *Duttonella* and *Trypanozoon*, the latter having the lowest infection rate of all known trypanosomes in vectors. The subgenus *Trypanozoon* has the most complex cycle of development and migration and at no time during this developmental cycle do parasites become associated with a cuticular epithelium. Killick-Kendrick *et al.* (1974) have suggested that this inability to associate with cuticle may be an explanation for the very low percentage of *Glossina* which become infected with these trypanosomes, and may be the reason for the complex developmental cycle observed. Certainly these parasites have an opportunity to adhere to mouthparts, as do *Duttonella* and *Nannomonas*

TABLE VII

*Summary of methods of attachment of trypanosomatid flagellates in invertebrate hosts*

| Species of parasite | Insect host | Sites of development | Method of attachment | Author |
|---|---|---|---|---|
| *T. (H.) lewisi* | *Nosopsyllus fasciatus* (flea) | rectum | hemidesmosome, insertion of flagella between rectal folds | Molyneux (1969a) |
| *Crithidia fasciculata* | *Anopheles gambiae* (mosquito) | colon (hindgut), rectum, rectal papilla | hemidesmosome | Brooker (1971a) |
| *T. (D.) vivax* | *Glossina fuscipes* (tsetse fly) | proboscis (labrum) | hemidesmosome | Vickerman (1973) |
| *T. (T.) brucei* | *Glossina* | salivary glands | insertion between microvilli and hemidesmosome | Steiger (1973) |
| *Herpetomonas muscarum* | *Chrysomyia chloropyga* | hindgut | hemidesmosome | Brun (1974) |
| *Leishmania mexicana amazonensis* | *Lutzomyia longipalpis* (sandfly) | midgut<br>oesophageal valve<br>pharynx | insertion between microvilli<br>hemidesmosomes<br>hemidesmosomes | Killick-Kendrick *et al.* (1974)<br>Molyneux *et al.* (1975b) |
| *T. (M.) melophagium* | *Melophagus ovinus* (sheep ked) | midgut<br>hindgut<br>rectum | insertion between microvilli<br>hemidesmosomes<br>hemidesmosomes | Molyneux (1975) |
| Trypanosomatid spp. | *Peromyscopsylla silvatica spectabilis* (flea) | hindgut | desmosome-like structure | Molyneux and Ashford (1975) |
| *Herpetomonas* spp. | *Culicoides variipennis* | malpighian tubules<br>midgut | insertion between microvilli, no hemidesmosomes seen | Hommel and Croft (1975) |
| *Le. b. brasiliensis* | *Lu. longipalpis* | hindgut,<br>hindgut triangle | hemidesmosomes | Killick-Kendrick *et al.* (1976b) |

species, as well as to the hindgut of *Glossina*, a site which the crocodile trypanosome *T. grayi* colonises in large numbers in *G. fuscipes* probably by the same method as *T.* (*M.*) *melophagium* adheres in *M. ovinus* hindgut (Molyneux, 1975). Thus, the inability to form attachment plaques in *Trypanozoon* cannot be due to the nature of *Glossina* cuticle. It is clear then that in some innate way this subgenus differs from the other trypanosomes which infect *Glossina* as it cannot attach to the cuticle or peritrophic membrane which is also of cuticular nature.

In many sites where parasites line the insect gut and produce a "pile carpet" the efficiency of the functional absorptive surface must be greatly impaired. This must apply in the hindgut and rectum rather than the foregut. The quantitative aspects of the effects of such parasitism on insect absorption would surely merit a study by insect biochemists and physiologists. The parasites themselves may be gaining from the close association, and a tendency to the reduction in size of the flagellar pocket as a result of the expansion of the intraflagellar area suggests that the specialised absorptive area of the parasite may be reduced. However, intake of complex molecules may occur through the pinocytotic-like vesicles that are observed where flagellar plasmalemma and cuticle come into contact. Such vesicles may be important in the nutrition of flagellates and have been seen in a variety of species attached in different hosts. The expansion of the flagellar membrane area, one of the commonest features observed when attachment occurs, is perhaps related to the need for as large as possible an area of contact between parasite and host to increase the efficiency of the adhesion (Curtis, 1972).

Parasites in large numbers in these sites seem to have some effect on the metabolism of vectors, as has been shown by Williams (1976). Ashford *et al.* (1973b) observed possible pathogenic effects of *Leishmania* in sandflies in the wild. Though this may have been associated with haemocoelic invasions, the commonest cause of pathogenicity in flagellate infected insects, destruction of midgut cells themselves, may be responsible (Garnham, 1955). Such changes of behaviour and metabolism should be more thoroughly studied in parasitised rather than in uninfected insects. It seems that the material and background knowledge available for future studies have been exploited on but few occasions, the notable one being by Watkins (1971a,b) using *Rhodnius prolixus* and *T.* (*H.*) *rangeli*. It seems regrettable that when so much information is available on the physiology of *Rhodnius prolixus*, a most important vector of human disease, nothing has been done by insect physiologists to study the physiological interaction between *T.* (*S.*) *cruzi* and the bug. This is the more regrettable considering that much of the justification for this work was based on the importance of *R. prolixus* as a vector of Chagas' disease. Clearly there is much scope for investigation in this field; the problem of working with *T.* (*S.*) *cruzi* can be overcome by using a less dangerous organism, *e.g. T.* (*H.*) *rangeli* or even a monogenetic flagellate system, as a model before further studies on the vectors themselves are made.

ACKNOWLEDGEMENTS

I am most grateful to many friends and colleagues who have contributed in

various ways in the preparation of this review. Professor Keith Vickerman, Drs Rolf Steiger, Reto Brun and Bob Killick-Kendrick, kindly allowed me to use illustrations and provided the negatives or prints. Drs Sherwin Desser and R. Khan kindly provided drafts of papers "in press". Drs Killick-Kendrick, Ashford, Hommel, Crewe, Croft and Professor W. Peters read part or all of the manuscript and I am most grateful to them for their most valuable suggestions. Finally, Miss Pat Dunmore and Mrs Diana Steedman assisted at all stages of the manuscript preparation and suffered my handwriting with admirable patience.

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# East Coast Fever: Some Recent Research in East Africa

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## I. Introduction

East Coast fever is a disease of cattle with considerable economic significance in the development of livestock industry in eastern Africa. The disease is a syndrome resulting from the infection of cattle by one or several of the protozoan parasites of the family Theileriidae indigenous to eastern Africa. It is almost invariably fatal, high-grade cattle (i.e. cattle of predominantly *Bos taurus* type) being particularly susceptible.

In 1967 the UNDP/FAO East African Livestock Survey published a report (Anon., 1967) in which it was concluded: "In East Africa, the tick-borne diseases are serious killers of cattle, and control of these diseases, above all East Coast fever, deserves the highest priority for disease research."

As a result of this report, the three countries of the East African Community —Kenya, Uganda and Tanzania—requested financial assistance from the United Nations Development Programme Special Fund to establish a research project aimed at producing a vaccine against East Coast fever. Supplementary requests were made for assistance to set up individual national projects, one of whose functions was to implement the findings of the regional project.

This review is based largely on the work carried out by the UNDP/FAO Immunological Research on Tick-borne Cattle Diseases and Tick Control Project, which became operational at the laboratories of the East African Veterinary Research Organisation in 1967, and on work carried out in collaboration with the Project. Unlike previous reviews of theileriasis by Neitz (1957) and Barnett (1968), it is thus largely restricted to research on the East African East Coast fever syndrome carried out during the last 7 years, and follows up a similar review by Wilde (1967).

## II. The Causative Organisms of East Coast Fever

The three parasites which, separately or in combination, are responsible for East Coast fever in East Africa are all members of the genus *Theileria*. The taxonomic position of the genus has always been a vexed question, and the classification used in the present text follows that made by the Committee on Taxonomy of the Society of Protozoologists (Honigberg *et al.*, 1964):

| | | |
|---|---|---|
| Subphylum | : | Sarcomastigophora |
| Superclass | : | Sarcodina |
| Class | : | Piroplasmea |
| Order | : | Piroplasmida |
| Family | : | Theileriidae |
| Genus | : | *Theileria* |

### A. *THEILERIA PARVA*

The parasite which has been regarded classically as the causative organism of East Coast fever is *Theileria parva* (Theiler, 1904). The type strain of the species commonly used for research purposes is *T. parva* (Muguga). It is a typical virulent strain, maintained in the laboratory for approximately 25 years. Its characteristics have been detailed by Brocklesby *et al.* (1961) and Brocklesby (1962b). The mortality rate of high-grade susceptible cattle when subjected to a classical 10-tick challenge has been reported as greater than 95% (Brocklesby *et al.*, 1961; Wilde, 1967).

*T. parva* is transmitted by the tick *Rhipicephalus appendiculatus*. Larval or nymphal ticks ingest the intra-erythrocytic piroplasm stage of the parasite whilst feeding on infected cattle. After the ticks have become engorged, they detach from the host animal and moult to the next instar. During this time, the parasite finds its way to the salivary glands of the ticks by a process which is not understood. When the tick attaches to a new host and begins feeding, the parasite undergoes a cycle of maturation resulting in the formation of infective particles which are inoculated into the host during the feeding process.

From the 4th day after inoculation of the infective particles, macroschizonts may be found in lymphoblasts in the lymph node nearest to the attachment site of the ticks. They increase in numbers throughout the lymphoid system approximately tenfold every 3 days until almost all of the lymphoid cells are parasitised. A characteristic febrile response, with the rectal temperature remaining above 39·5°C, begins as early as the 10th day after tick attachment and continues until the host dies. From the 14th day after tick attachment increasing numbers of macroschizonts give rise to microschizonts, host cells are destroyed, and micromerozoites are released and invade erythrocytes. Piroplasms, the intra-erythrocytic stages of the parasite, first appear about the 16th day after attachment and increase in numbers until the host dies, when more than 50% of erythrocytes may contain parasites. The hosts die from the 16th day onward, the degrees of parasitosis generally being proportional to host survival time.

### B. *THEILERIA LAWRENCEI*

*Theileria lawrencei* (Neitz, 1955) is a parasite whose importance in the East Coast fever syndrome is becoming increasingly realised. In this review, *T. lawrencei* is considered as a separate species from *T. parva* on the basis of recent cross-immunity studies on the two species cited by Burridge (1975). The validity of this separation is not universally accepted, and Levine (1971) classified *T. lawrencei* as a synonym of *T. parva*. A strain of *T. lawrencei* used recently for research purposes is *T. lawrencei* (Serengeti). The strain was isolated from captive buffalo in the Serengeti National Park, Tanzania, and its characteristics have been detailed by Young *et al.* (1973a). Adult *R. appendiculatus* ticks, fed as nymphs on the buffalo, transmit fatal infections to cattle. Development of the parasite in the salivary glands of the ticks appears to be similar to that of *T. parva*, although the infection rate of ticks is low. Individual infected ticks may, however, contain large numbers of infected salivary acini (Young and Purnell, 1973a). From about the 10th day after attachment of infected ticks, macroschizonts may be found in local drainage lymph nodes and they subsequently spread throughout the lymphoid system, although their numbers remain at a low level compared to those of *T. parva*. A febrile response begins on or about the 12th day after tick attachment. Microschizonts and piroplasms are either absent or scanty, and the host dies from the 16th day onward.

### C. *THEILERIA MUTANS*

*Theileria mutans* (Theiler, 1906) has long been regarded as a virtually ubiquitous non-pathogenic parasite of cattle in East Africa. Its involvement in the East Coast fever syndrome as a significant subsidiary pathogen has only just been determined. The strain of the species now used frequently in research is *T. mutans* (Aitong), which was isolated from cattle exposed at Aitong in the Narok District of Kenya (Irvin *et al.*, 1972d).

*T. mutans* has always been differentiated from *T. parva* and *T. lawrencei* by the fact that it is readily blood transmissible, the intra-erythocytic piroplasms

being capable of division and invasion of other erythrocytes. There is little evidence that the piroplasms of the other two species have this facility. Furthermore, until recently, there was no evidence that the East African *T. mutans could be transmitted by ticks. Irvin et al. (1972d) demonstrated that T. mutans* (Aitong) was readily blood transmissible, and caused high intra-erythrocytic parasitaemias in recipient animals often associated with anaemia resultant from destruction of erythrocytes by piroplasms. In early mechanical passages, recipient animals died, but, on continued passage, the virulence of the strain was apparently reduced. Young *et al.* (1976b) have now shown that *T. mutans* (Aitong) is transmissible by *Amblyomma variegatum* ticks, and have described the progress of infections produced in this manner, confirming the observation of Uilenberg *et al.* (1974) that *A. variegatum* is an efficient vector of *T. mutans.*

Tick transmission is trans-stadial, either larva to nymph or nymph to adult. There are indications that the transmission is less efficient than that of *T. parva* by *R. appendiculatus* (Purnell *et al.*, 1975a), infection rates being lower in ticks fed in their previous instars on hosts with comparable parasitaemias. The development of *T. mutans* in the salivary glands of *Amblyomma* is similar to that of *T. parva,* but the infective particles of *T. mutans* are large and *Babesia*-like.

From about the eighth day of attachment of infected ticks, macroschizonts are detected in the local drainage lymph node. They are morphologically distinct from those of *T. parva* or *T. lawrencei,* being larger, with larger and more numerous nuclei. Macroschizonts are also detectable in circulating mononuclear leucocytes. The macroschizont phase is transient and lasts about 6 days. Microschizont production begins on about the twelfth day. Parasites containing both micro- and macroschizont nuclei have been detected. Intra-erythrocytic piroplasms are also detected from the 12th day onwards. They divide in the blood by either binary or quarternary fission. The increase in piroplasm parasitaemia in tick-induced infections is quicker than that in mechanically-passaged blood infections. The parasitaemia may reach 40% by the twenty-first day after tick attachment. Infected animals do not normally undergo a febrile response. The main clinical feature of infection is an anaemia, during which the packed cell volume may drop to 12% by the twenty-sixth day. If the animal is going to survive the infection, and death from the infection is by no means inevitable, the parasitaemia falls from about this time, and the packed cell volume slowly returns to normal.

## III. The Development of the Parasites in the Tick Vectors

### A. *THEILERIA PARVA* (MUGUGA)

The cycle of development of *T. parva* in its common tick vector, *Rhipicephalus appendiculatus,* has remained incompletely known, mainly because investigators have been reluctant to study the development of the parasite in the engorged and moulting nymph. An engorging nymph ingests about $10^7$ piroplasms whilst feeding on a parasitaemic host; of these, 0–10 are likely to develop to the stages found in the salivary glands of the resultant adult tick. Despite the development of improved tracing techniques, such as radio-

labelling, the task is still daunting. Attempts have been made to trace parasites in the salivary glands of nymphal ticks on the verge of moulting to adults (Purnell, 1972, unpublished), but parasites have not been detected either in the glands or in muscle, as might be suspected from the work of Shortt (1936) on *Babesia canis*.

The development of the parasite in the salivary glands of adult infected ticks feeding on rabbits has been studied by Purnell and Joyner (1968). They found, in agreement with Cowdry and Ham (1932), that parasites can be detected in the salivary glands of unfed ticks, an observation that had been disputed by Reichenow (1940) and Martin *et al.* (1964). The first stage of the parasite seen was termed a "young form". These forms stained blue with Giemsa stain. Initially they appeared as a discrete mass, sometimes within a vacuole in the cytoplasm of the acinar cell. The outline of the parasite mass appeared to become more diffuse as the cytoplasm of the host cell was progressively occupied. At this stage nuclear hypertrophy in the host cell was sometimes very pronounced, and the presence of a greatly enlarged host-cell nucleus was often indicative of parasitisation. Young forms appeared to grow to occupy a large proportion of the host-cell volume.

The next stages of the parasite were categorised by Purnell and Joyner (1968) as "intermediate forms". They showed the first signs of nuclear differentiation with accompanying changes in the staining characteristics. The parasite mass appeared to be broken up into "blocks" or cytomeres in which particles of purple-staining material of varying size were present, probably being formed within them. In the final stages of this form, small discrete masses with more evenly spaced nuclear particles more uniform in size could be seen.

The final stage of the parasite was termed a "mature form". Here the parasites were finally differentiated into many discrete particles, each with a small purple-staining nuclear mass. These particles were also observed in the salivary ducts. Their formation appeared to coincide with disintegration of the host-cell nucleus.

Purnell and Joyner (1968) examined serial section of salivary glands of 561 ticks and classified the parasites they observed according to the above character-

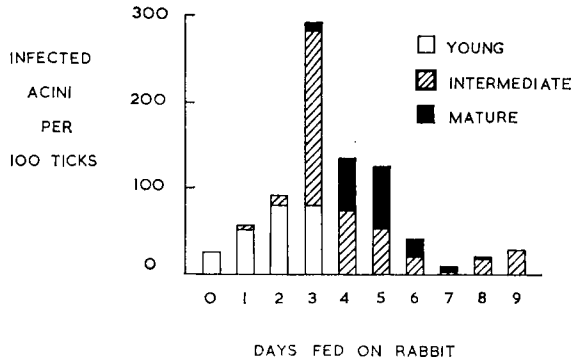


FIG. 1. The effect of duration of attachment of *Rhipicephalus appendiculatus* adult ticks upon the number of salivary acini observed, and upon the maturity of the parasites in the acini.

istics. Their results are summarised in Fig. 1. They observed that young forms predominated during the early stages of tick feeding on the rabbit host. As the incidence of young forms decreased, intermediate forms became numerous, with the peak of incidence of the parasites on the 3rd day of attachment. From this point the total numbers of infected acini diminished, but the highest numbers of mature forms were seen on the 4th and 5th days of tick feeding. A small number of parasites persisted during the 7th to 9th days of attachment, but these were mostly of the intermediate type and were thought to represent a minority of parasites which failed to reach maturity.

Purnell *et al.* (1971) investigated the comparative infection rates of adult and nymphal *R. appendiculatus* fed on, and dropping simultaneously from, a calf with a high *T. parva* intra-erythrocytic parasitaemia. They were investigating Reichenow's hypothesis (Reichenow, 1940) that if larval and nymphal ticks are fed on animals with the same level of infection, more parasite groups will be present in the salivary glands of the resultant nymphs than in those of the resultant adults. Reichenow also suggested that a group of nymphs, unlike a group of adult ticks, will begin to feed synchronously when applied to a host, and that the maturation of the parasite can be completed after 72 h feeding instead of 108 h as in adult female ticks.

Purnell *et al.* (1971) however, found that the adult ticks had a higher infection rate than the nymphs. In support of Reichenow's hypothesis, they did find that mature parasites were first seen in adult ticks after 5 days feeding, whilst they were seen in nymphal ticks after only 2 days. All parasites seen in nymphal ticks after 3 days feeding were mature. Furthermore, nymphal ticks were seen to feed steadily and synchronously after attachment, a regression line being calculable from the data of duration of attachment and mean body weight. *R. appendiculatus* adult tick feeding is known to be triphasic and somewhat asynchronous (Joyner and Purnell, 1968). Purnell *et al.* concluded that, in their experiment, there were approximately three times as many infected acini in adult ticks as there were in nymphs after synchronous engorgement of their previous instars. Since the weight of adult ticks containing mature parasites was approximately ten times the weight of the corresponding nymphs, it appeared that a batch of nymphs would contain more parasites per gram than a batch of adult ticks. They concluded that this fact, together with the uniformity of maturation of the parasites in nymphs, might well suggest that nymphal ticks should be preferred to adults for the preparation of ground infected tick stabilates.

It has been noticed frequently in the past that there is considerable variability in the infection rate of tick batches fed as nymphs on cattle with comparable parasitaemias. Thus, Lounsbury (1906) recorded that ticks, which fed in their previous instar on animals "in the blood of which the parasites were abundant", sometimes failed to infect susceptible oxen, whilst on other occasions ticks which fed as nymphs on oxen "in the blood of which no parasites were seen", infected susceptible oxen. Purnell *et al.* (1974d) correlated the results of the examination of salivary glands of laboratory-reared infected ticks with the intra-erythrocytic *T. parva* (Muguga) parasitaemias of the host animals on the day that the ticks dropped as nymphs. Their results summarised the examina-

tion of 1537 ticks during several years. Parasitaemias were in the range of 1%–50%. They showed that there was a significantly greater proportion of infected ticks in the range of parasitaemia 41%–50% than in the other ranges ($P < 0{\cdot}01$). In addition, when the data for the ticks fed on rabbits for 3 days were analysed separately it was found that the proportion of infected ticks in the range of parasitaemia 1%–5% was significantly lower than in the other ranges.

They then attempted to investigate the contribution of individual factors to the variation in infection rate. First, they established that adult ticks which had fed as nymphs on animals which had rising or peak parasitaemias were more likely to be heavily infected than those which had engorged on animals with falling parasitaemias. Second, they examined several batches of adult ticks which had dropped engorged from individual cattle at various stages during their fatal reactions. They showed that there was little correlation between the parasitaemias on the day of nymphal drop and the infection rate of the resultant adult ticks. Third, they inoculated a group of five cattle with aliquots of an infective stabilate and fed nymphal ticks on the cattle as they were undergoing similar reactions. The dissection of the resultant adult ticks revealed a complete range of infection rates in the five batches of ticks. Finally, a large batch of adult ticks, all of which had dropped engorged from a steer with a 29% parasitaemia, was examined. After 3, 4 or 5 days feeding on rabbits, groups of ten ticks were randomly selected, dissected, and had their salivary glands examined. In this way, 283 ticks were examined. The tick batch had a very high rate of infection. The salivary glands of the randomly selected ticks within the batch showed a considerable variation in infection rate regardless of days the ticks were pre-fed on rabbits. In some cases 9 or even 10 out of 10 ticks were infected, with a mean number of infected acini of 40–50; in another case as few as 4 out of 10 were infected, with a mean number of infected acini of less than 5. Purnell *et al.* (1974d) concluded that, since a very small proportion of ingested parasites survive, it may be that a purely physical factor will govern their survival. Such a factor, they suggest, could be the juxtaposition of developing salivary glands and gut epithelial cells during moulting. If the parasite migrates through the gut epithelium, the random contiguity of these tissues would assist in the migration of the parasite.

Advances which allowed the selection of highly infected tick batches (Purnell *et al.*, 1974d) and the feeding period after which there was a strong likelihood of the parasites being mature (Purnell and Joyner, 1968), made it practicable to attempt to visualise the fine structure of infective particles of *T. parva* in tick salivary glands despite the low acinar infection rate. Using salivary glands from 4-day-fed infected ticks dissected at EAVRO, fixed and flown on ice to Britain, Macmillan *et al.* (1971) found a mass of protozoa, which they believed to be infective particles, in an infected salivary acinus. Profiles of the infective particles measured up to 1·5 $\mu$m in diameter and appeared to be bounded by a single membrane. A prominent nucleus, up to about 1 $\mu$m in diameter, was present but no nucleolus was detected. Mitochondria were not present. Indications of an intratorial complex were present in the form of flask-shaped rhoptries (paired organelles). Electron-dense circular profiles, probably micronemes, were found

in all the infective particles. A further organelle was found and referred to as a "nestling body"; a small, usually circular, profile appeared to be situated in the concave indentation of a second kidney-shaped profile.

Scattered among the infective particles were larger bodies, up to 4·5 $\mu$m in diameter, which the authors thought probably corresponded to the cytomeres described by Martin *et al.* (1964) and Purnell and Joyner (1968). The cytomeres contained all the structures seen in the infective particles and, in addition, one example of a probable micropore was seen. Concentric membrane organelles were observed in some cytomeres. The fact that the cytomeres contained all the structures seen in the infective particles left the authors with the strong impression that the latter arose from the former.

The histological methods used by Martin *et al.* (1964) and Purnell and Joyner (1968) to detect parasites in tick salivary glands involve lengthy processing. Methods have now been evolved to rapidly assess tick infection rates. These methods are needed when partially-fed ticks are brought in from the field and are to be used in strain isolation or where a series of experiments is to be carried out on laboratory-infected ticks of a particular batch. Parasites in partially-fed ticks degenerate within one or two days if the ticks are removed (R. E. Purnell, 1969—unpublished), and it is well-known that the numbers of viable parasites in infected ticks gradually decrease with increasing age of the ticks (Martin *et al.*, 1964).

Kimber and Purnell (1972) devised a rapid technique for screening the salivary glands of a tick for parasites. The glands were removed under a suspension of G100 Sephadex to which gelatin had been added to 2·5%. Immediately after dissection the glands were frozen by placing them on a preformed block of gelatin-Sephadex in a cryostat. Sections were then cut at 5 $\mu$m, dried, fixed in methanol, stained with Giemsa's stain and mounted in DPX. The morphology both of normal salivary acini and of those infected with *T. parva* was shown to correspond very well with that observed after normal paraffin wax processing. Using the technique it was possible to assess the infection rate of a batch of ticks, and the maturity of the parasites during the course of a morning.

Kimber *et al.* (1973) modified this rapid method using fluorescent antibody techniques, with the eventual objective of assessing not only the infection rate of field-collected ticks but also the parasite strain with which they were infected. They tried two direct and one indirect method of fluorescent staining of sections of the salivary glands cut on the cryostat and fixed in acetone. The most convincing results were obtained using the indirect method, where bright fluorescence was demonstrated using serum showing a high titre to *T. parva* piroplasms. No fluorescence was exhibited using pre-infection serum from the same animal.

Blewett and Branagan (1973) recently described a technique for the demonstration of *T. parva* infection in intact tick salivary glands, and since no sectioning was required, it has advantages for application in the field. Glands were fixed in Carnoy's fluid and dehydrated overnight in absolute alcohol. They were then washed in hydrochloric acid, fixed in Feulgen's reagent, washed in sulphurous acid solution and processed through the alcohol series before final

clearing in xylol. The glands either could be examined in solid watchglasses containing xylol, using a stereoscopic microscope, or were transferred to microscope slides, broken up by gentle prodding with a fine needle and mounted. Feulgen-positive bodies were detected in these ways, and their incidence shown to coincide with the incidence of *T. parva* infection of the partnered salivary gland from the same tick processed by conventional means.

### B. *THEILERIA LAWRENCEI* (SERENGETI)

The development of *T. lawrencei* in the salivary glands of *R. appendiculatus* has been studied by Young and Purnell (1973b). Nymphal ticks were fed on either buffalo or cattle showing *T. lawrencei* piroplasms. The resultant adult ticks were dissected after feeding on rabbits for 3, 4 or 5 days, and their salivary glands were processed and examined for parasites. In both cases, the number of ticks showing parasites was low (5·9% from buffalo, 2·1% from cattle) and this may have been because the piroplasm parasitaemias in the hosts were low. However, several of the infected ticks had high individual infection rates. The morphology of the salivary gland stages of *T. lawrencei* could not be distinguished from that of *T. parva*, and mature parasites were detected after infected ticks had fed for 4 or 5 days, as was true for *T. parva*.

After continued passage through cattle, *T. lawrencei* undergoes a behavioural transformation (Young *et al.*, 1976c) and its infectivity for ticks is dramatically increased. Since the infection rate of ticks with the transformed strain is so high, it is used for comparative studies on the morphology of theilerial parasites in the tick vector (such as the work of Purnell *et al.* (1974e), which will be discussed in Section V).

### C. *THEILERIA MUTANS* (AITONG)

The development of *T. mutans* in the salivary glands of its common tick vector *Amblyomma variegatum* has been compared with that of *T. parva* in *R. appendiculatus* by Purnell *et al.* (1975a). Nymphal ticks of each species were fed on cattle reacting to infection with the appropriate parasite, and dropped engorged when the intra-erythrocytic piroplasm parasitaemia in each case was 40%. The resultant adult ticks were fed on rabbits, and on each of the first 5 days of feeding, male and female ticks were removed and their salivary glands dissected, processed and examined for parasites. No stages of *T. mutans* were seen in ticks until the ticks had fed for 3 days, while *T. parva* was seen even in unfed ticks. Mature stages of *T. mutans* were seen after the ticks had fed for 5 days, while the corresponding stages of *T. parva* were seen after 4 days. The *T. mutans* infection rate was consistently lower than the *T. parva* infection rate.

The development of *T. mutans* in the salivary glands could be distinguished from that of *T. parva*. The first stage of *T. mutans* observed was a compact morula which occupied part of one cell of a salivary acinus without distending it. This contrasts with *T. parva* where early stages generally occupy a large area of the host cell, often distending it and inducing nuclear hyperplasia. Intermediate stages of *T. mutans* observed were like those of *T. parva*, but the final, mature

stage differs from *T. parva* in that there appear to be fewer infective particles, which are definitely larger than those of *T. parva*. The large size of the infective particles of *T. mutans* is a feature of their cycle which gives the parasite an affinity to *Babesia*, a diagnostic feature mentioned by Young and Purnell (1973a).

## IV. Techniques for Infecting Ticks, and for Harvesting Parasites from them

In this section, the experiments described were carried out using *T. parva* (Muguga) and the laboratory strain of *R. appendiculatus*, unless otherwise stated. Branagan (1969) has shown that the infection of ticks with *T. parva* by feeding them on cattle reacting to laboratory-induced infections can best be achieved by applying nymphal ticks to cattle so that their engorgement coincides with the maximum intra-erythrocytic parasitaemia in their hosts. However, it is not always possible to induce and monitor theilerial infections under controlled conditions. Recently, methods have been evolved in which strains can be isolated in ticks from field cases of cattle already demonstrating intra-erythrocytic parasitaemias and rapidly approaching death. Joyner and Purnell (1968) demonstrated that Gregson's (1953) capillary tube feeding technique for *Dermacentor andersoni* could be modified and used to feed *R. appendiculatus* on a variety of blood substrates, and Purnell (1970) infected *R. appendiculatus* by feeding nymphal ticks on capillary tubes of blood or washed red cells taken from a parasitaemic steer. The nymphal ticks were first fed on rabbits for 4, 5 or 6 days until they were nearing engorgement, then removed from the rabbits and restrained on "Plasticine". They were then allowed to feed for a further 24 h on infected blood in capillary tubes. After 24 h they were removed from the "Plasticine" and allowed to moult through to adults. These were subsequently shown to be infective.

Although this method was suitable for isolating field strains, further experimentation resulted in techniques for the infection of large numbers of ticks. In a rather bizarre experiment, Irvin *et al.* (1970) fed nymphal ticks on a rabbit's ears until tick engorgement was nearly complete. They then killed the rabbit, cannulated the carotid arteries, severed the head and connected the cannulae into a perfusion system maintained by a flow pump. The perfusate used was defibrinated bovine blood with a high piroplasm parasitaemia, oxygenated using an aquarium pump. The system was run for 3 h and the severed head left for a further 24 h at 37°C. During this time, the majority of the nymphal ticks dropped engorged into the ear-bags enclosing the rabbit's ears, and were later shown to have given rise to infective adults, having completed their engorgement, at least in part, on the bovine blood perfusate.

Searching for a simpler method of infecting nymphal ticks feeding on rabbits, Irvin *et al.* (1974e) revived the technique of Brocklesby and Vidler (1962). The modified technique consisted of feeding nymphal ticks on rabbits and, either on 1 day or on several days towards the end of tick feeding, inoculating the rabbit intraperitoneally with 10–60 ml of heparinised infected bovine blood. Nymphal ticks subsequently dropped engorged and up to 10% of the resultant

adult ticks were shown on dissection to be infected. Further experimentation showed that it was possible to infect ticks by feeding the nymphs on rabbits inoculated with heparinised infected bovine blood stored at +4°C for up to 7 days. This suggested the practicality of the method for use in the isolation of field strains. Provided it was kept on ice, a Universal bottle of blood from a field case could be used to isolate a field strain several days' journey from the laboratory. In such a way, field strains from the Kiambu District of Kenya were isolated for subsequent cross-immunity trials, and cryopreserved as stabilates.

Purnell *et al.* (1974c) carried out a further series of experiments to try to find the best material to inoculate into the rabbits and the most favourable route of inoculation. Presence and persistence of infected bovine erythrocytes in the inoculated rabbits were monitored by taking blood smears from the rabbits at intervals after inoculation. Rabbits were either untreated, splenectomised, irradiated at different doses, or pre-treated by the inoculation of large amounts of bovine erythrocyte antigen. Infected blood was either heparinised or the erythrocytes were washed and resuspended in phosphate buffered saline, autologous plasma, or rabbit serum. Various quantities of inocula were given, and the intraperitoneal and intravenous routes of inoculation were used. Finally, it was shown that the intravenous inoculation of 20 ml of intact, heparinised, infected bovine blood into splenectomised rabbits gave the most consistent results. When nymphal *R. appendiculatus* ticks were fed on rabbits treated in this way, the resultant adult ticks showed high *T. parva* infection rates in their salivary glands.

The foregoing methods all rely on the infection of nymphal ticks during their final engorgement, but Purnell *et al.* (1970) have demonstrated that they do pick up infecting piroplasms during their early feeding. A more detailed study of the infections produced in ticks by feeding them on parasitaemic blood at particular stages in the feeding process might well give results which would improve indirect infection methods.

The capillary tube feeding technique for *R. appendiculatus* has provided the base from which several significant studies have emerged. Purnell and Joyner (1967) used the technique to collect mature infective particles from artificially-fed infected adult ticks. The ticks were fed first on rabbits for 4 days and then on capillary tubes of heparinised or defibrinated blood for 2 h. Inoculation of the residual feed from 20–26 ticks fed in this manner resulted in infection of 4 out of 11 recipient cattle. Brown *et al.* (1969) explained that this technique for harvesting infective particles was developed so that the residual infective feed pool could be titrated with the intention of finding a sublethal immunising dose for cattle. They found, however, that infective particles released into the blood in the tubes were immediately taken up by leucocytes, and could only become established in the white cell donor or its isogenic twin. Thus the possibility of immunising cattle using such a feed pool was discarded, and a search made for a substrate which could support the viability of the infective particles and also prove acceptable to all bovine recipients.

Cunningham *et al.* (1973b) carried out a series of experiments in which infected ticks, pre-fed for 4 days on rabbits, were fed for 2-h periods on capillary

tubes containing various substrates. The contents of the capillary tubes were subsequently inoculated into ECF-susceptible cattle. Heparinised plasma, serum and an erythrocyte plasma mixture were found to be unsuitable substrates since they did not support the viability of the infective particles, even when they were mixed with autocthonous leucocytes immediately after the 2-h feeding period and then inoculated back into the original donor steer. Precolostral calf serum, either a commercial preparation or prepared in the laboratory, foetal calf serum from an abattoir, and Eagle's Minimum Essential Medium particularly with the addition of 3·4% Bovine Albumin Powder (Fraction V from Bovine Plasma) all proved to be suitable substrates, since inoculation of cattle with feed pools of ticks fed on them resulted in death of the cattle from ECF. These substrates were therefore selected for subsequent experimentation.

Infected ticks restrained on "Plasticine" seemed to suffer no ill-effects and mated female ticks have been fed for several days to full engorgement (R. E. Purnell, 1968—unpublished). Joyner *et al.* (1972) have investigated the duration of emission of infective particles from ticks fed for 8 h whilst restrained in this manner. Two groups of 5 infected female ticks, pre-fed for 4 days on rabbits, were fed for four successive 2-h periods on capillary tubes of heparinised blood. Each blood feed pool was injected back into the original donor. In one case, the animal receiving the 0–2 h feed pool died of ECF whilst those receiving the 2–4 h and 4–6 h feed pools had ECF reactions; in the other case, the animals receiving 0–2 h and 2–4 h feed pools died of ECF and both other animals had ECF reactions. It was thus demonstrated that restrained female ticks will continue to emit infective particles for at least 6 h.

Purnell *et al.* (1969) modified methods used by Howell (1966) and Tatchell (1967) in an attempt to obtain pure saliva from infected ticks, expecting that the saliva thus obtained would contain a higher concentration of infective particles than substrate feed pools. They inoculated adult female ticks, previously fed on rabbits for 5 days, with representatives of the three groups of parasympathomimetic drugs (Goodman and Gilman, 1960). After a series of experiments they concluded that inoculation of 1 $\mu$l of either 10% pilocarpine nitrate or 10% arecoline hydrobromide in a 6% sodium chloride solution in distilled water using an Agla micrometer syringe (Burroughs Wellcome) gave the most consistent results. A greater proportion of ticks salivated, and the volume of saliva produced was highest when these drugs were thus used. Purnell *et al.* (1969) also found that larger, heavier, female ticks salivated more consistently than smaller ones, and produced more saliva. However, examination of the salivary glands of 5-day-fed infected female ticks in the different size ranges showed that there was an inverse relationship between the size of the partially fed ticks and their infection rates. In their final experiment Purnell *et al.* (1969) therefore selected 5-day-fed infected female ticks in a middle size and weight range. They inoculated 25 of these ticks with pilocarpine nitrate and collected the saliva in drawn-out capillary tubes placed over the ticks' hypostomes. The saliva was ejected into a bottle containing concentrated bovine leucocytes in phosphate-buffered saline as it accumulated over a period of 90 min. It was then injected into the leucocyte donor, which died of ECF 22 days

later. Infective material collected from ticks in this way has been used in a variety of attempts, as yet unsuccessful, to visualise and quantitate the infective particles (Purnell *et al.*, 1974b).

Artificial feeding of ticks has been used in preliminary attempts to introduce radio-isotopes into them. Purnell *et al.* (1972b) fed 5 groups of infected female ticks on rabbits for 2 days and then for 48 h on capillary tubes containing precolostral calf serum (PCS) labelled with $^{3}$H-thymidine (5–20 $\mu$Ci/ml). They were then fed briefly on tubes of clean PCS and finally for 3 h on further tubes containing clean PCS. This PCS was filtered through a 0·45 $\mu$m Millipore filter and counted using a liquid scintillation counter. The mean count-rate of Millipore filters from infected ticks was significantly higher ($P<0{\cdot}005$) than that of filters from uninfected ticks. This suggested that the infective particles secreted by ticks were labelled by this means.

In recent studies we have also been able to introduce $C^{14}$-glycine and $Ce^{144}$ into engorging female ticks whilst feeding them on foetal calf serum or heparinised blood containing solutions of the radio-isotopes (Purnell *et al.*, 1974a). As briefly described by Hyland and Hammar (1959), the larval ticks hatching from the eggs laid by these female ticks have been radiation-labelled. Sonenshine (1968) has shown that such ticks may be of considerable value in ecological studies.

Infective particles harvested from infected ticks fed on capillary tubes of foetal calf serum have also been used to infect bovine lymphoid cells in culture. Such cells can then be used in studies on the early stages of the parasite in the bovid, which appear to be the critical stages in immunogenic procedures. Brown *et al.* (1973) established monolayers of cells, reared from calf mesenteric lymph nodes, in small plastic T flasks using a mixture of Eagle's Minimum Essential Medium and 20% foetal calf serum. After maintenance for 2, 9 and 23 days, the supernatant was discarded and the cultures were inoculated with either *in vitro* tick feed or ground tick supernatant, each culture receiving material from the equivalent of 15 ticks of the same infected batch. After inoculation all the cultures were overlaid with fresh growth medium and incubated at 37°C. The medium in all cultures was changed once a week. Infected lymphoid cells were observed 26 days after inoculation in the 9 and 23 day cultures. Cell lines were established from these transformed cultures.

The primary objective in developing an artificial feeding technique for *R. appendiculatus* was to harvest infective particles for titration in cattle, but there were soon indications that an alternative method was simpler and could be used for larger numbers of ticks. Cunningham *et al.* (1973c) harvested parasites from infected male or female ticks, previously fed for 4 days on rabbits, by feeding the ticks on capillary tubes of heparinised blood for a period of 2 h. The blood feed pool was then inoculated back into the blood donor. Groups of 5 cattle were thus inoculated, each with the *in vitro* feed pool from either 50 male or 50 female ticks. After being fed on the capillary tubes, each group of ticks was removed from the "Plasticine" and ground in a Griffith's tube with 10 ml of Eagle's Minimum Essential Medium containing 100 units of penicillin and 100 $\mu$g of streptomycin per ml. Each ground tick suspension was inoculated into a steer. The results are shown in Table 1. There was some doubt about the ECF

TABLE I

*Infection rates in ECF susceptible cattle inoculated with either* in vitro *feed or ground tick suspension from male and female* R. appendiculatus *ticks infected with* T. parva (*Muguga*)

| Sex of ticks | Inoculum | No. of susceptible cattle | No. of cattle infected |
|---|---|---|---|
| female | *in vitro* feed | 5 | 5 |
| | ground tick suspension | 4 | 4 |
| male | *in vitro* feed | 3 | 1 |
| | ground tick suspension | 4 | 3 |

susceptibility of four of the recipient cattle; when these were excluded, as in Table I, the results indicated that female infected ticks yielded a more consistent harvest of infective particles, but that a ground tick suspension was as infective as an *in vitro* feed.

The technique for harvesting infective particles by grinding pre-fed infected ticks was improved by subsequent experiments, and the method adopted was described by Purnell *et al.* (1973a).

The experiment they described was designed to select the most suitable day on which to prepare a *T. parva* stabilate, and to see if observations on the morphology of the parasites in the salivary glands of the vector tick could be correlated with their infectivity for cattle. They selected a batch of ticks known by previous salivary gland examination to be infected and fed 10 groups of 50 male and 50 female ticks on rabbits. At daily intervals a total of 50 male and 50 female ticks were removed at random from several rabbits. Half the ticks were then dissected, processed, and examined for parasites, whilst the remainder were ground in Eagle's Minimum Essential Medium (MEM Grand Biological Company, Grand Island, New York, USA) supplemented with 3·5% Bovine Albumin Powder (BPA Fraction V from Bovine Plasma, Armour Pharmaceutical Company Ltd., Eastbourne, England), and soda glass. After standing for 1 h in a measuring cylinder, the supernatant fluid was pipetted off and aliquots inoculated into susceptible cattle. The results showed that no parasites capable of infecting cattle were produced until the ticks had fed for 4 days. The most acute ECF reactions were produced in cattle inoculated with material from ticks fed for 5 days. The results of salivary gland examination showed that no mature parasites were observed in ticks fed for less than 4 days. The greatest number of mature acini were observed in ticks fed for 4 days, female ticks containing more infected acini than male ticks. In a subsidiary experiment, Purnell *et al.* (1973a) selected small, medium and large 5-day-fed infected female ticks, dissecting some and grinding others. Aliquots of supernatant fluids from all three groups of ticks killed all inoculated cattle. Mature parasites were seen in ticks of all three weight ranges, although significantly more were seen in the medium-sized and large ticks than in the small ticks. The results showed, therefore, that there was a good correlation between morphology and infectivity of

parasites, and that supernatant fluids of high infectivity are best prepared from 5-day-fed female ticks regardless of their degree of engorgement.

With the realisation that *T. lawrencei* has a considerable importance in the East Coast fever syndrome, it also became necessary to prepare highly infective *T. lawrencei* stabilates for vaccination attempts. An experiment was carried out on *T. lawrencei* by Young *et al.* (1975) to complement that done by Purnell *et al.* (1973a) and discover the most appropriate feeding period for infective ticks before stabilate production. The strain of *T. lawrencei* used was known as *T. lawrencei* (KB5), derived from an orphan male buffalo kept in captivity at EAVRO. The strain was under consideration as a potential vaccine "master" strain. In a preliminary experiment it was determined that the infection rate in the ticks to be used was rather low, so a total of 4650 ticks, fed as nymphs on the buffalo, were applied to rabbits. At that time, and at daily intervals for 7 days, 175 male and 175 female ticks were removed, 50 of each sex for salivary gland examination and 125 of each sex for preparation of ground tick supernate. Aliquots representing one-tenth of each supernate were inoculated into each of 3 cattle daily. The reactions of the cattle are summarised in Table II. The results indicated that very highly infective stabilates were prepared from ticks fed for 5 or 6 days. Examination of the tick salivary glands indicated that the greatest numbers of mature parasites were present in ticks fed for 5 days, followed by ticks fed for 6 days. Ten per cent of the tick salivary glands examined were infected, and the number of infected acini observed in individual infected ticks was low. Nevertheless, prepatent periods as short as 4 days were observed in 3 animals, indicating both a high infectivity of individual parasites and an efficient method of harvesting them.

The most critical step taken in the utilisation of parasites harvested from ticks was their subsequent cryopreservation as stabilates (Lumsden and Hardy, 1965) for standardising infection of cattle. Cunningham *et al.* (1973a) described methods for the cryopreservation of both *in vitro* tick feed pools and ground

TABLE II

*The reactions of cattle to inoculation with aliquots of supernatant fluid prepared from ticks infected with* T. lawrencei *fed for varying periods of time on rabbits*

| Duration of tick feeding in days | Mean prepatent period in days | Mean time to febrile response in days | Mean time to death in days |
|---|---|---|---|
| 0 | — | — | — |
| 1 | — | — | — |
| 2 | — | — | — |
| 3 | 11·3 | 15·0 | — |
| 4 | 6·3 | 10·7 | 16·7 |
| 5 | 4·7 | 10·0 | 15·0 |
| 6 | 4·3 | 9·3 | 14·3 |
| 7 | 6·7 | 10·7 | 14·0 (2/3) |
| 8 | 7·3 | 11·0 | 16·0 |
| 9 | 7·7 | 10·0 | 14·7 |

tick supernates. In their first experiment groups of pre-fed female infected ticks were fed on capillary tubes of precolostral calf serum (PCS) for 2 h, and the resultant feed pool divided and either inoculated directly into cattle or diluted with glycerolised PCS to a final glycerol concentration of 7·5% (v/v). Some of the glycerolised aliquots were then inoculated into cattle whilst others were placed in bijou bottles, wrapped in cotton-wool, and placed at −80°C in a deep-freeze (Revco Ultra Low Deep freeze, Model ULT 661, Revco Inc., Deerfield, Michigan, U.S.A.). The following day the frozen aliquots were rapidly thawed by immersing the bottles in a water batch at 37°C and then inoculated into cattle. The mean times to death of cattle inoculated with untreated and frozen *in vitro* feed were equal (17·3 days).

In a second experiment, the feed pool was glycerolised by drop-wise addition of glycerol to 7·5% and then dispensed in 30 × 0·1 ml aliquots to glass capillary tubes. The tubes were wrapped in cotton-wool in a cardboard container, frozen slowly to −80°C overnight and then transferred to gas-phase liquid nitrogen (−196°C). After 192 days storage, the capillary tubes were rapidly thawed, the contents diluted to 1:50 with glycerolised foetal calf serum, and 10 cattle each inoculated with 1 ml of diluted stabilate (representing material from 1·6 ticks). All the cattle developed patent ECF, and 7 died.

Cunningham *et al.* (1973a) also ground 2000 pre-fed female infected ticks and collected 150 ml of supernate, which was glycerolised to 7·5% by the addition of an equal volume of foetal calf serum containing 15% glycerol. The supernate was then dispensed into 120 × 2·5 ml aliquots in glass tubes, the tubes being placed in a container of 1-in. thick expanded polystyrene and then slowly cooled to −80°C. Over the next 441 days of storage, 1 ml aliquots (representing material from 6·7 ticks) inoculated into susceptible cattle killed all of them.

In a final experiment 465 ml of material was obtained from 10 000 pre-fed infected ticks, slowly cooled to −80°C in 2·8 ml aliquots and then transferred to gas-phase liquid nitrogen. Over the next 340 days of storage, 1 ml aliquots (representing material from 21·5 ticks) inoculated into susceptible cattle killed all of them.

As a preliminary demonstration of the value of stabilate material produced in this way, Cunningham *et al.* (1973d) compared the reactions produced in ECF-susceptible and ECF-recovered cattle either when inoculated with aliquots of stabilate (representing material from approxiately 15 ticks) or when a classical 10-tick challenge was administered (Bailey, 1960). The ECF-recovered cattle were a group which had fortuitously recovered from the inoculation of a diluted *T. parva* stabilate 1 month previously. The ticks used, either for application to the ears of the cattle undergoing challenge or for stabilate production, were all from the same batch, of confirmed infectivity.

All the ECF-recovered cattle resisted both forms of challenge, whilst all the susceptible cattle died. The mean time to death of cattle receiving a 10-tick challenge was 20·3 (±2·35) days, and of those receiving stabilate was 15·5 (±2·37) days. It was thus demonstrated that cattle recovered from stabilate infection could resist a natural tick challenge, and that uniform reactions could be produced by stabilate inoculation. The advantages of using stabilate material were pointed out: (1) Reactions are reproducible over an extended period; (2)

Infections are always lethal, compared with those produced by Brocklesby *et al.* (1961) where doubt existed as to the infectivity of ticks in 25 out of 153 cases; (3) Since no ticks are applied, the challenged animals do not have to be so strictly confined as those to which a 10-tick challenge is given.

Recently, further improvements have been made to techniques of stabilate production (C. G. D. Brown and M. P. Cunningham, 1974—pers. comm.). Scottish sand, washed first in sulphuric acid and then in distilled water at pH 7·0, is preferred to soda glass for grinding since use of the latter results in a high pH in the suspension. Sedimentation of deposit is carried out by centrifuging the material at 50 g (50 ml tube) or 100 g (100 ml tube) for 5 min. The aliquots of material are stored in a roll of 3 mm corrugated cardboard and are then frozen at approximately 1°C per min to −80°C in the Revco. These changes appear to have resulted in improved stabilate infectivity.

Purnell *et al.* (1971) suggested that nymphal ticks might be preferable to adults as a source of infective stabilate material, since there were more parasites per gram of nymphal material than of adult material when the ticks had been fed synchronously on an infected animal in their previous instars. There also appeared to be a more uniform maturation of parasites in the nymphs. Purnell *et al.* (1974e) carried out the comparative study using a cattle-passage strain of *T. lawrencei* (Serengeti) which, as mentioned previously, is highly infective for ticks.

Larval and nymphal ticks were fed synchronously on each of two steers inoculated with a *T. lawrencei* stabilate, whose disease reactions could be anticipated. The ticks dropped engorged from the steers when intra-erythrocytic parasitaemias were high. The ticks were allowed to moult, and were then fed on rabbits for 3 days in the case of nymphs or 5 days in the case of adults. These feeding periods had been determined previously to allow the maximum number of parasites to mature (Purnell *et al.*, 1971). After removal from the rabbits, the tick batches were divided, and some ticks were processed so that the infection rate in their salivary glands could be assessed, whilst others were

TABLE III

*Reactions of susceptible cattle inoculated with different volumes of* Theileria lawrencei *(Serengeti) stabilate prepared from adult and nymphal ticks*

| Origin of stabilate | "Tick equivalent" of inoculum | Mean prepatent period in days | Mean time* to febrile response in days | Reaction† | Mean time* to death in days |
|---|---|---|---|---|---|
| adult ticks | 50 | 7·2 | 10·2 | 5D | 16·8 |
| | 5 | 8·2 | 12·0 | 5D | 17·6 |
| | 0·5 | 11·8 | — | 3D 2R+R | — |
| nymphal ticks | 50 | 7·6 | 11·4 | | 19·0 |
| | 5 | 9·2 | — | 1D 4R+R | — |
| | 0·5 | 15·2 | — | 1D 4R+R | — |

* calculated only where applicable to all animals in group

† D = died; R+R = reacted and recovered

processed to produce stabilates (Cunningham *et al.*, 1973a). The stabilates derived from the nymphal and adult ticks were each titrated in cattle. Groups of 5 cattle were each inoculated with the material derived from the equivalent of 50, 5 or 0·5 ticks. The reactions of the cattle are shown in Table III. The results supported the view that the prepatent period of cattle infected with *Theileria* is related to the infective dose (Jarrett *et al.*, 1969a), as will be discussed in the next Section. They indicated, therefore, that at each dose level significantly less infective material was derived from nymphs than from adults. This result was not supported by the results of examining the tick material, where it was seen that the nymphs contained at least as many parasitised acini as the adults. The conflict in the results may have been either because each infected nymphal acinus contained fewer parasites or because the parasites were not mature in the nymphs although they appeared so. To prove the latter point it would be necessary to carry out the preparation of sequential daily stabilates from nymphs. The experiments showed that it was much easier to obtain an even suspension by grinding nymphs, and since other investigations are under way on feeding very large numbers of larvae all over the body of an infected animal, it may well be that stabilates prepared from nymphs fed as larvae on infected animals will eventually be preferred. A more careful assessment of parasite maturity may lead to the production of more highly infective stabilates.

## V. The Development of the Parasites in the Bovine Host

The life-cycle of *T. parva* in the bovine host has been well documented, and recently acquired knowledge of it has been discussed by Wilde (1967). With the exception of the early invasion of the host, the successive stages have been studied in some detail.

The current review deals with recent studies on the development of the parasite with special reference to those directly leading to an ECF vaccine. In this context, the study of the kinetics of replication of *T. parva* by Jarrett *et al.* (1969a) had a considerable influence on the techniques of quantitative assessment of the effects of parasite invasion adopted by subsequent workers.

Jarrett *et al.* (1969a) induced infections of varying severity in susceptible cattle by applying 10, 100 or 1000 ticks from an infected batch to one of their ears. The parotid lymph node regional to the infested ear was then palpated daily until it became enlarged, after which needle biopsies of the right prescapular lymph node were carried out daily. Thin films prepared from these biopsies, and thin blood films from a sectioned superficial ear vein, were stained with Giemsa's stain and examined for schizonts and intra-erythrocytic piroplasms respectively. A minimum of 400 erythrocytes was examined per blood film and the number containing piroplasms was recorded as a percentage. Lymph node films were examined (1) to obtain the macroschizont index (MSI) and (2) to classify the macroschizonts according to the number of nuclei contained within them. The MSI was estimated by selecting an area of the film at random and making a linear count of at least 400 lymphoid cells and their associated macroschizonts, both intracellular and extracellular. The result was expressed as a percentage. A minimum of 100 extracellular macroschizonts was examined

in each film to determine the distribution of macroschizont nuclear numbers. Since Jarrett *et al.* also dissected cattle and determined their mean total lymphoid count, MSI's could be discussed in terms of the total number of macroschizonts present in an animal.

In the first experiment, where 10 ticks were fed on each animal, small numbers of macroschizonts were found on day 16 after tick attachment and the geometric means of the MSI of days 17–20 were 2·9, 16·4, 27·3 and 30·3. In the second experiment, where 100 ticks were fed, geometric means of the MSI of days 14–17 were 5·3, 13·5, 33·6 and 52·4. In the third experiment, where 1000 ticks were fed, the values for days 11–14 were 2·5, 4·5, 12·7 and 21·0. In each case a linear relationship between the logarithm of the MSI and time could be demonstrated. The growth rate of the parasite could be calculated from the slopes of the regression line, and was expressed as the $T_{10}$. Thus, for *T. parva* (Muguga), the slope of the regression lines was found to be 0·33, and the $T_{10}$, which was defined as the time in days for a ten-fold growth of the parasite, was 3 (the reciprocal of the slope). The mean number of lymphocytes in the animals used was estimated to be $2{\cdot}4 \times 10^{12}$, and the total number of macroschizonts on any one day could therefore be calculated by multiplying the MSI for that day by $2{\cdot}4 \times 10^{10}$. Extrapolation of the modified data suggested an input of $10^{6{\cdot}7}$ parasites for 10 ticks, and this was in agreement with figures obtained by reference to other work on the tick stages of the parasite (Martin *et al.*, 1964; Purnell and Joyner, 1968). The days on which the febrile response first occurred were noted and the means for each group compared with the calculated macroschizont numbers on those days. The results coincided well between the groups and indicated that the mean number of macroschizonts which had been produced when fever occurred was $7 \times 10^9$.

Counts of the number of nuclei per extracellular macroschizont showed that distributional peaks occur in the macroschizont population on any given day. Early in the disease, most contained 1–6 nuclei. Later the number in the 6–12 category increased, and later still there was an increase in those with larger numbers of nuclei. It was demonstrated that the percentage of a particular category of macroschizont was approximately the same on any given day in the three groups of animals, although the actual number of macroschizonts might vary enormously. This suggestion of a time-dependency in the course of the disease was further substantiated by the observation that intra-erythrocytic piroplasms occurred only after the finding of macroschizonts with more than 12 nuclei on day 16 or 17. The illustration that the switch from the exponential phase of replication to the maturation phase when macroschizonts began to form microschizonts was time-dependent, suggested that it took place after the parasites had undergone a fixed number of divisions.

Jarrett *et al.* (1969a) used their results to illustrate the differences between the pathogenesis of different strains of *Theileria*. A 10-tick challenge with a strain having a $T_{10}$ of 1·5 would be expected to have similarities with a 1000-tick challenge with *T. parva* (Muguga) where the $T_{10}$ is 3. They pointed out that the 50% immunising dose is between $10^8$ and $10^9$ macroschizonts as shown in their vaccination experiments (Pirie *et al.*, 1970), and that it is probable that a period of about 3 weeks is required before an animal becomes immune.

Where a strain has a $T_{10}$ of 5, immunity would probably start to influence the clinical picture resulting from infection, and at a $T_{10}$ of 7 the disease would never surface clinically but the animal would be immune to further challenge. Where $T_{10}$ was 10, Jarrett *et al.* (1969a) suggested that a considerable scatter of the population about this mean figure would occur, resulting in a situation where small numbers of piroplasms were produced over an extended period but schizonts were not detectable by normal methods. They suggested that diagnosis of *T. mutans* infection may be made in such a situation, and that an intermediate situation might exist in cases diagnosed as *T. lawrencei*. They also used their data to illustrate how the "natural immunity" of some breeds of cattle could result in a decrease in the rate of replication of a strain of parasite so that a normally lethal 10-tick challenge with a $T_{10}$ of 3 resulted in an immunising dose when the $T_{10}$ became 4. They pointed out that the "natural immunity" could be broken down by increasing the tick challenge, which explained results obtained by Barnett (1956).

Finally, Jarrett *et al.* (1969a) used their data on the numbers of piroplasms seen in erythrocytes to show that the frequency of multiple invasion of erythrocytes fell within normal limits of a Poissonian distribution and that multiple invasion did not necessarily imply multiplication of piroplasms within erythrocytes.

The techniques and terms used in this study have now come into routine use, and the quantitative interpretation of the life-cycle of *Theileria* has assisted in the choice and assessment of experiments designed towards production of an ECF vaccine. With the development of techniques for harvesting parasites from ticks, as described in the previous section, the opportunity to extend these experiments was presented.

There were several problems encountered by Jarrett *et al.* (1969a). First, it was very difficult for them to make a logical sequential assessment of the dynamics of growth of the parasite since parasites were being continually introduced by the feeding ticks. Second, they were unable to ensure that the parasite input from one group of 10 ticks would remotely resemble the input from another, even when the ticks dropped engorged as nymphs from the same animal on the same day. This has been illustrated by Purnell *et al.* (1974d) and discussed in Section III. Since Jarrett *et al.* were also restricted to the use of small groups of cattle, there could well be considerable variation within groups. Finally, since they were restricted to the introduction of parasites by feeding ticks and no more than 1000 ticks could feed on an animal, they could only feed groups of 10, 100 or 1000 ticks. Where parasites are harvested from a batch of ticks as described by Purnell *et al.* (1973a), a much greater variation in infective input is possible.

The experiments carried out by Radley *et al.* (1974) made use of the new techniques. In the first experiment, 4300 ticks were selected from batches proven to be infective by prior dissection of samples of ticks from them using the techniques described by Purnell and Joyner (1968). The ticks were ground after feeding for 4 days on rabbits, and a total supernate of 132 ml was collected. Two animals were each inoculated with 64 ml and two with 0·1 ml. Their reactions were observed using the parameters enumerated by Jarrett *et al.* (1969a). Mean regression lines of MSI against days after inoculation were

plotted for each group. The $T_{10}$ for the animals receiving 64 ml inocula was 1·41 and for those receiving 0·1 ml was 4·93. This suggested that the growth rate of the parasite in its bovine host might well be dose-dependent and is not a character of the strain.

A second experiment was carried out to investigate this possibility. A total of 3950 ticks from infected batches were fed on rabbits for 4 days and then ground. The supernate was made up to 320 ml, and then pairs of cattle were inoculated with either 0·1 ml, 1 ml, 10 ml or 100 ml of the suspension. The results are shown in Table IV. The shortest prepatent period observed was 5 days. Animals which received 100 ml of supernate had considerable lymph node hyperplasia on the 3rd and 4th day after inoculation, but no macroschizonts were detectable on extensive examination of biopsy smears from them despite being common in such smears on the 5th day after inoculation.

TABLE IV

*The reactions of four groups of cattle to inoculation with suspensions of* Theileria parva *tick-derived supernates (Expt 2)*

| Group | Volume of inoculum given to each animal in ml | Animal number | Prepatent period in days | Time to febrile response in days | Time to detection of piroplasms in days | Reaction* (time to death in days) |
|---|---|---|---|---|---|---|
| 1 | 100 | C 675 | 5 | 6 | — | D (11) |
| | | C 762 | 5 | 6 | — | D (11) |
| 2 | 10 | C 674 | 6 | 7 | 12 | D (13) |
| | | C 718 | 6 | 7 | 13 | D (13) |
| 3 | 1·0 | C 664 | 6 | 7 | 13 | D (15) |
| | | C 666 | 6 | 7 | 13 | D (18) |
| 4 | 0·1 | C 670 | 8 | 7 | 13 | D (24) |
| | | C 761 | 7 | 7 | 13 | D (19) |

* D = died

The only obviously differing feature of the reactions of the animals in various groups was the time taken for them to die. This time increased linearly from a mean of 11 days (Group 1) to a mean of 21·5 days (Group 4). Mean regression lines of MSI against days after inoculation were again plotted for each group. In each case, the slopes of the regression lines, and thus the growth rates of the parasites, differed significantly from each other. A linear relationship between the $T_{10}$ value and the log infective dose was demonstrated.

A final experiment was carried out in which larger groups of cattle were used, and which included a group where a further reduction in infective dose was made. A total of 7350 ticks were ground to give a suspension of 460 ml. Groups of four cattle were each inoculated with the following suspensions: 100 ml, 10 ml and 1 ml of undiluted supernate, 1 ml of supernate diluted 1:10, 1 ml of supernate diluted 1:100. The reactions of the inoculated cattle are shown in Table V. The results showed that the prepatent period, the time to febrile

TABLE V

*The reactions of five groups of cattle to inoculation with suspensions of* Theileria parva *tick-derived supernates (Expt 3)*

| Group | Volume of inoculum given to each animal in ml (dilution) | Animal number | Prepatent period in days | Time to febrile response in days | Time to detection of piroplasms in days | Reaction* (time to death in days) |
|---|---|---|---|---|---|---|
| 1 | 100 | D 348 | 5 | 6 | 13 | D (13) |
| | | D 360 | 5 | 7 | 13 | D (13) |
| | | D 365 | 5 | 7 | 13 | D (13) |
| | | D 368 | 5 | 7 | 13 | D (16) |
| 2 | 10 | D 349 | 6 | 8 | 13 | D (14) |
| | | D 351 | 6 | 7 | 13 | D (18) |
| | | D 352 | 6 | 9 | 12 | D (14) |
| | | D 376 | 5 | 10 | 12 | D (13) |
| 3 | 1 | D 347 | 6 | 11 | 13 | D (14) |
| | | D 366 | 6 | 9 | 13 | D (15) |
| | | D 377 | 6 | 7 | 12 | D (18) |
| | | D 379 | 7 | 9 | 13 | D (16) |
| 4 | 1 | D 354 | 7 | 9 | 13 | D (20) |
| | (1 : 10) | D 358 | 8 | 13 | 13 | D (17) |
| | | D 372 | 7 | 9 | 13 | D (22) |
| | | D 374 | 7 | 9 | 13 | D (15) |
| 5 | 1 | D 346 | 13 | 16 | 13 | MR |
| | (1 : 100) | D 361 | 9 | 13 | 14 | D (19) |
| | | D 364 | 8 | 11 | 14 | D (24) |
| | | D 370 | 9 | 10 | 13 | MR |

* D = died; MR = mild reaction

response, and the time to death, increased as the number of parasites inoculated decreased, but the time to appearance of piroplasms remained constant. Regression lines of the altering features of the animals' reactions were plotted. The plotting of mean regression lines of MSI against days after inoculation confirmed the results of the first two experiments.

The results obtained by Radley *et al.* (1974) confirm those of Jarrett *et al.* (1969a) in showing that piroplasm production is time-dependent, not dose-dependent. The results of the two groups of workers are at variance in their most important aspect relating to the eventual production of a vaccine. Jarrett *et al.* (1969a) suggested that the growth rate of any particular strain of parasite would be constant in susceptible cattle and, thus, that vaccination might be possible using a strain of *Theileria* having a suitably slow growth rate. Radley and his co-authors suggested that any strain of *Theileria* would be a suitable vaccine strain, provided it is used at an appropriate dosage level. They illustrated their suggestion by reference to the work of Cunningham *et al.* (1974a), which will be discussed in the next section. They emphasised that their results did not suggest that the growth rate of *T. parva* (Muguga) varied directly with the

infective dose used, but rather that a combination of factors, dependent largely on the reaction of the recipient animal, acted to a greater or lesser extent on the multiplying parasites; these factors either eventually destroyed the parasites or failed to contain them, as a result of which the animal died.

Radley and his co-authors were unable to detect macroschizonts in lymph node biopsy smears before the 5th day after inoculation, even when massive infective doses were used. Since they were able to show that the relationship between prepatent period and infective dose was otherwise linear, they surmised that there is another stage of the life-cycle of *T. parva* interpolated between the infective particle and the uninuclear primary macroschizont in a lymphoid cell, as suggested by Wilde (1967). Radley *et al.* (1974) suggested that this unknown stage in the life-cycle of the parasite may be disguised either by being associated with the host cell nucleus, by having a different staining reaction to the conventional macroschizont, or by being present in a different site or in a limited focus within the lymph node.

The development of techniques to label infective particles (Purnell *et al.*, 1972b) and to observe the action of infective particles on bovine lymphoid cells *in vitro* (Brown *et al.*, 1973) and *in vivo* (Johnson, 1974) may soon lead to further knowledge of this stage of the life-cycle.

## VI. Vaccination

### A. Titration of infective dose

Until recently, attempts to vaccinate cattle against ECF have progressed little from the heroic efforts of Spreull (1914), who attempted to immunise 280 000 cattle by the intravenous administration of suspensions of spleen and lymph glands from infected animals. Twenty-five per cent of the animals died after this treatment, but 70% of the survivors were completely or partially immune. Brocklesby *et al.* (1965) revived this method of vaccinating cattle, using rather more sophisticated techniques, and demonstrated that recovered animals had considerable resistance to a 10-tick challenge. Pirie *et al.* (1970) further explored the method, by harvesting macroschizont-infected leucocytes in lymph nodes removed from killed infected donor animals. Cattle were then vaccinated by the intravenous inoculation of suspensions containing $10^8 - 10^{10}$ macroschizonts. Two doses of $10^9$ macroschizonts with an interval of 3 weeks between inoculations appeared to be most successful, protecting 8 of 10 animals against a subsequent tick challenge.

The difficulties in obtaining and quantitating macroschizonts from donor animals discouraged subsequent experimentation using bovine-derived parasites, and the development of simple methods of harvesting and preserving large numbers of parasites from ticks, as described in Section V, obviated the necessity to continue on this line. In preliminary attempts to use dilution of live tick-derived parasites, Cunningham *et al.* (1974a) found that they were unable to obtain uniform reactions in cattle given apparently identical aliquots of infective material. In the final experiment in their series a stabilate was prepared by feeding pre-fed female ticks on capillary tubes containing foetal calf

serum and preserving the harvested feed pool as described previously (Cunningham *et al.*, 1973a). Several capillary tubes of the stabilate were thawed 6 months after preservation, pooled, and diluted to 1/50, 1/150 and 1/450 using glycerolised foetal calf serum. Groups of ten cattle each were then inoculated with 1 ml of each dilution, and their reactions observed. Seven out of 10 inoculated with the 1/50 dilution died, as did 4 out of 10 receiving the 1/150 and 1 out of 10 receiving the 1/450 dilution. Surviving animals had a variety of reactions, and it was clear that vaccination using a titrated dose of live material would not be acceptable. The results of these experiments suggested that harvesting infective particles either by the *in vitro* feeding technique or by grinding infected ticks, did not yield a suspension in which the parasites were evenly dispersed. Dilution of the suspension did not therefore result in a successful titration. This problem had previously been encountered by Wilde *et al.* (1968), who found on one occasion that the inoculation of suspensions containing infective material from the equivalent of 1 or 3 ticks produced no reactions in susceptible cattle, material from 0·1 tick equivalents resulted in an ECF reaction, whilst material from 0·01 and 0·001 tick equivalents caused fatal ECF reactions.

### B. IRRADIATION

Cunningham and his colleagues turned then to the possibility of modifying the course of the infection produced in cattle by sublethally damaging the parasite either in its tick host, its free infective particle stage, or in the recipient bovine host. Their initial attempts to do this relied on the well-known fact that $^{60}$Co-irradiation can produce attenuated forms of parasites, suitable for vaccination.

Purnell *et al.* (1972c) first investigated the effects of $^{60}$Co-irradiation on the vector tick, *R. appendiculatus*. In a series of experiments, they established that the viability of unfed nymphal ticks and of nymphal ticks irradiated 2 h after engorgement was seriously affected by doses of irradiation in excess of 2 krad. Nymphal ticks irradiated mid-way through their moulting period, and unfed adult ticks were far more tolerant. Irradiation of moulting nymphal ticks at doses up to 16 krad had no effect on their subsequent moult, although the ability of resultant male ticks to attach to a host was seriously affected by previous irradiation at doses of 8 krad and above. Irradiation of adult ticks at doses up to 16 krad had no effect on their subsequent ability to attach to a host, although doses above 1 krad had detrimental effects on the fertility of female ticks and the virility of males. It appeared from these experiments that the most appropriate time to irradiate the vector ticks to modify the parasites without affecting the feeding of the ticks, would be when the ticks were unfed adults.

Purnell *et al.* (1972a) then carried out a morphological examination of the development of *T. parva* in the salivary glands of feeding adult ticks irradiated at various doses before feeding. Their results showed that irradiation at doses as low as 0·5 krad appeared to reduce the numbers of parasites developing in infected ticks' salivary glands, but that parasites were still observed in infected ticks irradiated at 32 krad. Doses of 4 krad and above appeared to alter the

morphological development of the parasite, and agglomeration of parasite masses in ticks receiving high doses of irradiation suggested that the viability of the parasites might be severely affected.

Completing the investigation of the effects of irradiation on the parasite in the tick vector, Purnell *et al.* (1974f) carried out three experiments in which infected ticks are various stages in their life-cycle were irradiated across the range of doses they were able to tolerate. Recently engorged nymphs were irradiated at 1, 2 and 4 krad, the latter failing to moult. When adult ticks resultant from the moulting of the 1 and 2 krad-irradiated nymphs were fed on rabbits and their salivary glands examined for parasites, those parasites which did develop were seen to be morphologically normal. This was also the case with adult ticks resultant from the irradiation of moulting ticks at doses up to 16 krad. In a preliminary experiment on irradiation of adult ticks, batches of 10 ticks were applied in ear-bags to cattle after irradiation at doses of 4–32 krad, and parallel batches of ticks were applied in ear-bags to rabbits. The ticks on the cattle were allowed to engorge, but those on rabbits were removed after 4, 5, 6, 7 or 8 days' feeding, and their salivary glands were then examined for parasites. In this instance, it was seen that the results obtained by Purnell *et al.* (1972a) were a partial misinterpretation of the effects of irradiation. In the earlier paper, salivary glands were examined after the ticks had fed for 3 or 4 days, and it was concluded that irradiation reduced the number of parasite masses developing in infected ticks. The results of the later study showed that increasing doses of irradiation slowed down the development of the parasite masses, thus, the greatest number of infected acini were seen in unirradiated ticks after 4 days' feeding, and in ticks irradiated at 32 krad after 7 days' feeding.

In the final experiment, infected unfed adult ticks were irradiated at doses of 5 to 60 krad. They were then fed on rabbits for 5, 6 or 7 days, applied so that all the ticks in a particular irradiation group were removed from rabbits on the same day. Ticks were either dissected and their salivary glands removed, processed, and examined for parasites, or ground as described previously (Purnell *et al.*, 1973a). Aliquots of the suspensions obtained by grinding the ticks were inoculated into groups of five cattle. Increasing doses of irradiation resulted in increasing numbers of ticks dying when applied to rabbits and also in decreasing numbers of morphologically normal parasites being seen in the salivary glands of dissected ticks. The destruction of the parasites was reflected in the reactions of the cattle receiving suspensions of tick material. As the irradiation dose increased, so the cattle had increasingly mild reactions, and animals receiving material from ticks irradiated at doses above 20 krad, with one exception, had no reaction. The exception was an animal which received material from ticks irradiated at 45 krad; it had an extended prepatent period, but nevertheless died of typical ECF. This anomalous result, together with the variation in the results obtained in animals receiving material from ticks irradiated at 20 krad (one had a severe reaction, three had mild reactions, one had no reaction and was susceptible on challenge), discouraged the authors from optimism regarding this method of producing a vaccine. Furthermore, since the safety limits were demonstrably too narrow (cattle dying after re-

ceiving 15 krad material, and not reacting to 25 krad material), it was concluded that the method did not offer great prospects of success.

Cunningham *et al.* (1973e) investigated the effects of irradiation on infective particles harvested from vector ticks by the methods of Purnell and Joyner (1967). Three experiments were carried out, and, in the final one, suspensions of infective particles in foetal calf serum were irradiated at doses of 4 to 32 krad. After irradiation, aliquots of the suspensions were inoculated into groups of five cattle. Once again, inoculation of material receiving increasing doses of irradiation resulted in reactions of decreasing severity in the cattle. The critical dose appeared to be 13 krad. Animals receiving material irradiated at 10 krad died or had ECF reactions, whilst four our of five animals receiving material irradiated at 16 krad had no reactions and were susceptible on challenge. Of the animals receiving material irradiated at 13 krad, only one had an ECF reaction, but three others were nevertheless immune to the subsequent lethal challenge. Whereas the reactions of the other groups of cattle had suggested that irradiation was merely resulting in a titration effect, i.e. increasing doses of irradiation killed increasing numbers of organisms, this result suggested that attenuation of the parasites might be taking place. However, as was the case when the parasites were irradiated in the tick vector, the safety limits were so narrow that the authors were deterred from further investigation.

### C. CHEMOPROPHYLAXIS

Since it appeared that the parasites could not be attenuated by irradiating them either in their tick vector or when they were in free suspension, attention was turned to the possibility of suppressing the parasite after its introduction into a susceptible host. Some success had been achieved previously in this respect by feeding infected ticks in ear-bags on the animals and by daily oral administration of chlortetracycline hydrochloride or oxytetracycline hydrochloride for 28 days after infestation, as was noted by Brocklesby and Bailey (1965) who reviewed the earlier work. Jarrett *et al.* (1969b) had demonstrated more recently that the oral administration of chlortetracycline hydrochloride in this way would suppress the overt symptoms of ECF if administered for 16 days but not if only continued for 8. Their calculations, based on allied quantitative studies on *T. parva* macroschizonts (Jarrett *et al.*, 1969a), indicated that administration of the drug for 16 days had suppressed the multiplication of the parasite so that an immunogenic effect was produced, coincident with the presence of approximately $10^9$ macroschizonts, but no pathogenic effect, as is observed when the macroschizont numbers build up to $10^{10}$.

Until recently the principal disadvantage in the infection and treatment method of vaccinating cattle has been the need to give extended drug treatment. Since the development of improved techniques for harvesting parasites, however, the opportunity exists for giving cattle a unique inoculation of infective material thus obviating the need for extended drug treatment, resulting from the gradual release of infective particles by feeding ticks.

Radley and his co-workers have recently produced a series of papers in which they have shown that the infection and treatment method can be used as an

economically viable method of vaccinating cattle against ECF for exposure throughout East Africa. In the first paper (Radley *et al.*, 1975a) two experiments were described. In the first of these, 25 cattle were immunised against *T. parva* (Muguga) by inoculation of 1 ml of an infective stabilate and concomitant treatment with experimental formulations of oxytetracycline drugs. Although the treatment required was not specified, it generally took the form of four consecutive daily inoculations of 5 mg/kg of the particular experimental formulation. The animals survived the immunisation procedure, generally with mild ECF reactions, and were then challenged by the inoculation of a stabilate of one of four recently isolated strains of *T. parva* or of a strain of *T. lawrencei* derived from buffalo but passaged through cattle. A parallel series of susceptible control cattle was inoculated with the stabilates. The immunised animals had minimal reactions to challenge with two of the *T. parva* strains, which were apparently homologous to *T. parva* (Muguga), but significant reactions to challenge with the other three strains. However, the reactions of the immunised animals to the heterologous challenges were not as severe as the reactions of the control cattle. None of them died, whereas 23 out of 25 susceptible animals died. In the second experiment, groups of cattle were immunised against the same five strains of *Theileria* by stabilate infection and four consecutive daily inoculations of 5 mg/kg of a polyvinyl-pyrrolidone based formulation of oxytetracycline, starting immediately after infection. They all had mild or inapparent reactions during the immunisation procedure. One month later they were all challenged with a lethal dose of the isologous strain of the parasite, and only two demonstrated parasites. After another month they were challenged with a lethal dose of a *T. parva* (Muguga) stabilate and all survived without apparent reaction. The results of these two experiments thus indicated that animals could be immunised by stabilate infection and four daily treatments of an oxytetracycline drug against a variety of theilerial strains. They were then protected against isologous challenge or challenge with *T. parva* (Muguga). Immunisation against *T. parva* (Muguga) did not invariably protect against all theilerial strains, but did confer at least some protection against all strains used.

Since it appeared that *T. parva* (Muguga) would not be suitable for a monovalent infection and chemoprophylaxis vaccine, Radley *et al.* (1975c) turned to another strain of *Theileria* as a candidate vaccine strain. This was *T. lawrencei* (KB5) a strain of *T. lawrencei* isolated from a buffalo captured as a very young orphan on a farm in Kenya and subsequently held captive at EAVRO. Thirty cattle were immunised against this strain by inoculation of 1 ml of a 1 in 10 dilution of a stabilate and six consecutive daily inoculations of 10 mg/kg of a propylene glycol-based formulation of oxytetracycline. The drug/parasite regimen was thus orientated towards greater suppression of the parasite, and only two of the animals demonstrated macroschizonts on a single occasion. However, all the animals reacted serologically. They were then challenged either with the isologous strain, a homologous strain, one of three other *T. lawrencei* strains, or a strain of *T. parva* known to break through *T. parva* (Muguga)-induced immunity. The cattle were protected against challenge with the identical or related *T. lawrencei* strains, but were only partially pro-

tected against a strain of *T. lawrencei* from another area of East Africa, and against the *T. parva* strain. As in the former trial (Radley *et al.*, 1975a) the survival rate of immunised cattle was very much higher than that of parallel groups of susceptible cattle receiving the stabilates. Only one immunised animal died compared to 21 out of 30 susceptible cattle. The authors concluded that *T. lawrencei* (KB5) would not be suitable as a monovalent vaccine strain, but suggested that a "cocktail" of different theilerial strains might well be used to vaccinate animals for field exposure in a variety of situations in East Africa.

The possibility of using a "cocktail" of three theilerial strains for infection and chemoprophylaxis vaccination of cattle against ECF was explored by Radley *et al.* (1975d). In the first of two experiments, 10 cattle were immunised by infection with 1 ml of *T. parva* (Muguga), 1 ml of a strain of *T. parva* known to break through *T. parva* (Muguga) immunity (*T. parva* Kiambu 5), and 1 ml of a strain of *T. lawrencei* originally isolated from a buffalo but now altered in many of its characteristics by passage through cattle (*T. lawrencei* Serengeti transformed). The infection was followed by four consecutive daily inoculations of 5 mg/kg of a formulation of terramycin (Terramycin Q-Pfizer Corporation). Other animals were immunised against one of two elements of the "cocktail" separately. The immunised animals were then challenged with either *T. parva* (Kiambu 1) or *T. lawrencei* (KB1), both strains known to break through *T. parva* (Muguga) immunity. The animals immunised against the "cocktail" of strains had mild or inapparent reactions to challenge, whilst susceptible control animals and many of those immunised against one or two of the constituent strains either had severe reactions or died. In the second experiment, cattle were immunised by inoculation of the same "cocktail" of strains and a single injection of 10 mg/kg of an experimental oxytetracycline formulation immediately after infection. They all had severe reactions subsequent to the immunisation procedure, since the single inoculation of drug had not sufficiently suppressed the parasite. These animals were then challenged with normally lethal doses of stabilates of strains recently isolated in Uganda and on the Kenya Coast, several hundreds of miles away from the places of origin of the "cocktail" constituents. They had mild or inapparent reactions to challenge, whilst 13 of 15 susceptible control cattle died.

Radley and his co-workers concluded from this series of experiments that infection and chemoprophylaxis using a "cocktail" of theilerial strains and a limited drug regimen was an effective means of vaccinating cattle against a variety of heterologous East African strains of theileriosis.

A possible criticism of the work carried out by Radley and his co-workers was that immunised cattle were only challenged by the single inoculation of a normally lethal dose of a tick-derived infective stabilate. The situation faced by immunised animals in the field would be different, since they would be continuously challenged with infective particles liberated into their blood stream during the course of feeding of successive infected ticks. The situation in the field was therefore reproduced by Purnell *et al.* (1975b), who infected susceptible cattle with lethal doses of a *T. parva* (Muguga) stabilate, seeded larval and nymphal *R. appendiculatus* ticks on them so that their engorgement would

coincide with the appearance of intra-erythrocytic piroplasms, and then released the cattle into a double-fenced five-acre paddock. The ticks engorged, dropped, moulted, and soon built up a highly infected tick population in the paddock. This population was sustained by the introduction into the paddock of successive groups of susceptible cattle, which became infected and which died after infecting further ticks themselves. When the infected tick population in the paddock had reached a high level, Radley *et al.* (1975b) introduced into the paddock a group of five animals, immunised against *T. parva* (Muguga) by infection and treatment. Five ECF susceptible cattle were introduced at the same time, and observations of tick population build-up and disease reactions of the two groups were made. Theilerial macroschizonts were demonstrable in lymph node biopsy smears from all animals after a prepatent period of 9–15 days. In the susceptible animals febrile responses occurred coincidentally and the parasitaemias increased until the animals died 16 to 20 days after exposure, but in the immunised animals, the parasitaemias were transient and no fever or other pathogenic effect occurred. Two further groups of susceptible cattle were consecutively exposed together with the immune animals, and they all succumbed rapidly to ECF. After 60 days exposure, the immunised animals were removed, having resisted challenge by up to 1300 potentially infective adult ticks at any one time. These results, together with a parallel exposure of *T. lawrencei*-immunised cattle in a *T. lawrencei*-seeded paddock (A. S. Young, 1974—pers. comm.), show that vaccination by infection and chemoprophylaxis will protect cattle against very severe homologous field challenge.

Although the duration of immunity produced in vaccinated cattle has not yet been investigated, it seems reasonable to expect that it will coincide with that produced after natural recovery. Burridge *et al.* (1972) have recently demonstrated that this is in excess of three and a half years, although they noticed that there was an increase in the number of animals demonstrating parasites with increasing time, showing that the immunity did eventually wane.

Recent work in other areas of East Africa has suggested that pathogenic strains of *T. mutans* such as *T. mutans* (Aitong) isolated by Irvin *et al.* (1972d) may be present over a wider range of East Africa than was thought previously. It may well be that an immunogenic "cocktail" acceptable throughout East Africa will have to contain a *T. mutans* element. The isolation and characterisation of *T. mutans* (Aitong) in its common tick vector *Amblyomma variegatum* (Purnell *et al.*, 1975a) has led to the production of *T. mutans* (Aitong) tick-derived stabilates which will shortly be included in infection and treatment cross-immunity trials. The search for a long-acting formulation of oxytetracycline which will suppress the parasite by a unique injection immediately after inoculation of the infective stabilates, is still continuing. Precise quantitation of immunogenic doses of parasites and their relation to the accompanying drug regimen is also being investigated. Two possible disadvantages of the infection and treatment method of vaccinating cattle against the East African East Coast fever syndrome are that it has been necessary in the past to give more than one inoculation of drug, and that, on occasions, due to insufficient suppression of the parasite, a carrier state may have been induced in immunised

animals. This means that a very few parasites have been allowed to complete their life-cycle to the intra-erythrocytic piroplasm stage, which may be infective for feeding ticks. Intermittent occurrence of such piroplasms in the blood of immunised animals has suggested that the use of inadequately suppressed alien strains may provoke epizootic outbreaks of ECF throughout East Africa. With continued research on parasite/drug ratios, both of these problems should soon be overcome.

### D. TISSUE CULTURE

In parallel with the development of the infection and chemoprophylaxis method of vaccination, studies have continued on the possibility of producing a tissue culture vaccine against ECF. Such a vaccine might well be more acceptable since it could be safer and easier to administer.

Undoubtedly one of the most significant breakthroughs in ECF research in recent years was the successful *in vitro* cultivation of bovine spleen cells infected and transformed by *T. parva*, as reported by Malmquist *et al.* (1970). The opportunity was thus created for extensive studies on the possibility of producing a tissue culture vaccine against ECF, either by titrating tissue culture macroschizonts or by modifying them using a variety of techniques. Spleen cell lines from calves experimentally infected with *T. parva* were established by growing them in Eagle's Minimum Essential Medium with Earle's salt base together with 20 % foetal calf serum. Spleen cells became attached to the surface of the T-flask culture vessel and formed confluent monolayers in 5 to 7 days. Up to the formation of monolayers, there were numerous cells in suspension and loosely attached to the surface, having the appearance of lymphoblasts. Forty-eight hours after implantation of the spleen tissue, approximately 75 % of the cells in suspension contained macroschizonts. Beginning at 7 to 10 days after implantation, there was a gradual decrease in the number of cells in suspension and an apparent disappearance of parasitised cells. For several weeks, the cultures had the appearance of non-infected spleen cells. After 5 to 6 weeks, they developed numerous foci of rounded cells in the monolayers. The rounded cells eventually detached from the monolayers and floated individually or in clumps of cells in the medium. There was a concomitant destruction of the monolayers so that the overall effect resembled the cytopathic action of a virus. By subculturing only the cells in suspension, a cell line of actively motile lymphoblasts was produced, in which 90 % to 95 % of the cells were parasitised. The cell lines thus established were maintained in the laboratory for many months subsequently. The plating efficiency of the cell lines was greatly increased when the cells were implanted on preformed monolayers.

Malmquist *et al.* (1970) observed that cell transformation was undoubtedly initiated by the parasite, which also stimulated lymphoblast multiplication. Moulton *et al.* (1971a) investigated this phenomenon further by light and electron microscopy, India ink carbon labelling, treatment of cultures with actinomycin D, and autoradiography. They showed that spleen cultures from infected animals transformed from macrophages to reticulum cells to lympho-

blasts that grew in suspension culture and had nearly 100% infection with *Theileria*. India ink carbon labelling was used to prove reticulum cell origin of lymphoblasts, and actinomycin D was shown to have a considerable inhibitory effect on the transformation of the reticulum cells. Moulton *et al.* (1971a) concluded that *T. parva* was a potent stimulus for lymphoblast formation and that the cell system they described was an excellent model for following all stages of lymphocytogenesis *in vitro*.

In another paper (Moulton *et al.*, 1971b) the same authors studied the growth characteristics of *T. parva*-infected bovine lymphoblast cultures. They demonstrated that, during 4-day growth cycles, the infected lymphoblasts had a six-fold increase in cell number. The macroschizont nuclei reacted positively for deoxyribonucleic acid with acridine orange stain, and the macroschizont cytoplasm had specific immunofluorescence with fluorescent antibody. The macroschizont nuclear number remained constant, usually 10 to 14 per cell, and the nuclei aligned themselves along the mitotic spindle fibres during cell division. The nuclei were often distributed equally in the daughter cells. Tritiated thymidine was incorporated in the lymphoblast nuclei, but not in the parasites.

Since Malmquist *et al.* (1970) had observed that plating efficiency of cell lines was greatly increased by implanting the cells on preformed monolayers, in a subsequent series of experiments Malmquist and Brown (1974) attempted to establish lines aspirated from *T. parva*-infected bovine lymph nodes using bovine embryo spleen (BESP) cells as preformed feeder layers. Their attempts were successful in four out of five cases, but parallel attempts to produce subculturable cell lines without feeder layers were only successful in one instance. They suggested that the technique should have practical value in the establishment of new cell lines infected with different strains of *Theileria*, since it interfered minimally with other experimental procedures.

*T. lawrencei* has also been successfully cultivated *in vitro* with material obtained directly from the host and cultured on BESP feeder layers. Stagg *et al.* (1974) described a simple technique for harvesting leucocytes from a buffalo calf by the sedimentation of heparinised blood at room temperature for 1 h. Approximately 10 ml of leucocyte-rich plasma could be harvested from each 20 ml of blood. This plasma was centrifuged at 180 *g* for 5 min and the pellet containing leucocytes was resuspended in culture medium and placed on a BESP feeder layer. After 18 to 42 days in culture, transformation occurred and the cultures gave rise to cell lines of lymphoblastoid cells infected with macroschizonts. On chromosomal analysis, the infected cells were shown to be of buffalo origin.

Brown *et al.* (1971) reported on experiments designed to see whether the inoculation of lymphoid cell culture infected with *T. parva* macroschizonts could induce theileriosis in recipient cattle. They used the first three tissue culture lines of *T. parva* (Muguga) isolated by Malmquist *et al.* (1970). They found that cattle could be infected by the subcutaneous or intravenous inoculation of $10^7$ or more tissue culture lymphoblasts containing macroschizonts. The infection which developed in cattle following injection of such material was not confined to the macroschizont stage of the parasite as is the case of

*T. annulata.* Once established in the cells of the recipient animal, the parasite completed its bovine life-cycle, with macroschizonts, microschizonts, micromerozoites, and intra-erythrocytic piroplasms observable. The piroplasms proved to be infective for nymphal *R. appendiculatus* ticks, and the resultant adult ticks induced a classical ECF reaction in cattle on which they were fed. The inoculation of $10^9$ infected cells was shown to produce fatal theileriosis in two cattle, the post mortem picture in both animals being typical of the response caused by *T. parva* (Muguga). A preliminary titration of the infective material was carried out, and it was seen that the majority of cattle which received inoculations of $10^5$ to $10^9$ infected lymphoblasts underwent mild or inapparent reactions followed by increased *T. parva* antibody titres. The cattle which reacted in this way to inoculation were subsequently shown to be resistant to a normally lethal tick-induced challenge.

Early optimism engendered by the preliminary series of experiments was not sustained by the results of later experiments. Thus, it was seen that groups of cattle inoculated with aliquots of infected lymphoblasts did not all react with equal severity to the infection. Of ten cattle, it was likely that one would not react, eight would react with varying severity but recover, and one would die. There was a strong suggestion that the varying reactions were related to the affinity between the lymphocyte grouping of the recipient animal and of the original lymphocyte donor (C. G. D. Brown, 1971—pers. comm.). No benefit was gained immunogenically by inoculating two doses of cell culture schizonts. Since it was clear that virulent infective material would not provide an acceptable vaccine, attempts were made to attenuate the parasite. C. G. D. Brown (1974—pers. comm.) has carried out prolonged *in vitro* cultivation but, although *T. parva* (Muguga) does appear gradually to lose its pathogenicity, this is accompanied by a corresponding loss in immunogenicity. Release of schizonts from host lymphoblasts by physical or chemical means has also been attempted but has not yet led to improved immunogenicity.

Turning to irradiation as a possible means of attenuating the parasite, Irvin *et al.* (1975a) irradiated aliquots of *T. parva*-infected lymphoblasts at doses of 300–1200 rad. They then evaluated the short-term effects of irradiation by examination of Giemsa-stained smears and by autoradiography of cells labelled with $^3$H-thymidine. Irradiation inhibited cell division but parasite division did not appear to be inhibited, and increases in macroschizont nuclear particle number of up to 50 times the normal value were recorded.

There was no evidence of an increased percentage switch from macro- to microschizont. Increasing doses of irradiation resulted in corresponding decreases in the numbers of viable cells observed, although this effect did not become pronounced until 3 to 4 days after irradiation. Apparently viable cells were, however, still present in all cultures 4 days after irradiation. A preliminary experiment on the inoculation of irradiated tissue culture into cattle has been carried out (C. G. D. Brown, 1974—pers. comm.) and there were indications that increasing doses of irradiation simply killed increasing numbers of host lymphoblasts. There was no evidence of attenuation of parasites.

## E. ATTEMPTS TO ESTABLISH *T. PARVA*-INFECTED TISSUE CULTURE MATERIAL IN SMALL ANIMALS

Another possible means of attenuating *T. parva* might be its adaptation, either partly or entirely, to an abnormal host. This is well-known to have been a successful method of producing vaccines against other diseases. In previous reported work infecting other hosts with *T. parva*, the attempts have been made by applying infected ticks to the potential host, by inoculating it with infected tick salivary glands, with infected bovine blood, or with an emulsion of infected bovine spleen. These attempts have been summarised by Brocklesby and Vidler (1961a, 1962) and have only been successful in that, on occasion, nymphal *R. appendiculatus* ticks have become infected while feeding on the animals into which blood has been injected. The application of this method of using the host as a vessel for infecting ticks has been discussed in a previous section. With the successful cultivation of *T. parva* in bovine lymphoblasts, the opportunity of further exploring its adaptation to other hosts was created. A concentrated suspension of infected lymphoblasts could be easily prepared. The material could be easily quantitated, and its infectivity and growth parameters readily defined. Parasitised cell lines could be cryopreserved, and thus a particular challenge could be reproduced using fresh or revived material.

Irvin *et al.* (1972c) inoculated *T. parva*-infected bovine lymphoid cells into Swiss albino male mice previously irradiated at 900 rad using a $^{60}Co$ source. Mice inoculated with $6 \times 10^7$ cells, of which 82% contained macroschizonts, began to develop palpable masses at the site of cell inoculation from day 7. By day 10 all the mice had developed such masses, which were shown on post mortem examination to be subcutaneous, firm, white and tumour-like. They were seen to infiltrate into the muscles of the shoulder and neck. Mice were killed in random pairs at intervals of 2 days. Initially the masses were avascular but towards the end of the experiment a small number of infiltrating blood vessels were observed. Histologically the lesions showed many features associated with malignant neoplasia of lymphoid cells.

Examination of impression smears from the cut surface of the masses showed that the cells present were almost entirely lymphoid and that a high percentage were parasitised. As the experiment advanced there seemed to be progressive breakdown of these lymphoid cells with a consequent increase in the number of extra-cellular macroschizonts, and occasional microschizonts were seen. Parasitised lymphoid cells undergoing mitosis and cell division were noted throughout the experiment.

The tissue masses from the two mice killed on day 12 were dissected out, weighed (total weight = 1177 mg) and finely chopped with scissors; the resultant cell suspension contained $1{\cdot}5 \times 10^7$ cells, 27% of which contained macroschizonts. This suspension was then put on to a bovine embryo spleen feeder layer (Malmquist *et al.*, 1969). The remaining material was trypsinised for 2 h at room temperature and a further $5{\cdot}1 \times 10^7$ cells obtained. From this suspension $1{\cdot}0 \times 10^7$ cells were then injected into each of five mice irradiated at 900 rad 7 h earlier. Both attempts to re-isolate infected material were successful. The parasitised cells became re-established in tissue culture and one of the mice

developed a parasitised tumour-like mass at the site of inoculation. There was no evidence that the parasite was growing in cells other than those of bovine origin.

The extension of this work was reported in a further paper (Irvin *et al.*, 1974b). The dose of irradiation given to the mice before inoculation was varied, and 800–900 rad shown to result in production of the largest tumours. The size of the tumours was recorded on palpation using a scoring system, and tumour size shown to reach a peak at day 12, declining slowly thereafter until they disappeared by day 44. The histopathological changes in the mice were observed, and seen to consist of an infiltration of lymphoid cells into muscle and fat at the site of inoculation, resulting in a progressive destruction of muscle fibres. After day 17, host response supervened and the subsequent changes within the tumour-like masses were largely degenerative. The mean schizont number in macroschizonts from impression smears was seen to decline gradually up to day 8 and then to return to the original level in the inoculum. There was once again no evidence that mouse cells became parasitised.

Attempting to explore the possibility of growing the infected bovine lymphoid cells in other systems, Irvin *et al.*, (1972a) inoculated cells into normal mice and newborn mice by the subcutaneous route, and also into hamster cheek pouches and the anterior chamber of rabbits' eyes. There was no evidence of growth or persistence of parasitised cells in any of these systems, nor were tumour-like masses formed.

Irvin *et al.* (1974d) then inoculated aliquots of infected bovine lymphoid cells into mice of a variety of strains after the mice had been irradiated at 710 rad. Seven strains of mice were used. The mice were examined daily after inoculation, and inoculation sites were palpated. The size of any tumour-like mass developing was recorded, and its duration also noted. It was possible then to give a figure for the mean size and duration of masses, calculated by scoring the sizes and dividing the sum of the daily means by the numbers of days on which masses were detected. The information thus obtained indicated that the tumour-like masses grew better in the A2G strain of mice than in any other strain used, and it was decided to use them for further experiments,

One major problem with all conventional strains of mice was that the tumour-like masses were rejected by the host. Irvin (1975) attempted to overcome this by various techniques for immunosuppression of the host response. The use of such hosts as neo-natally thymectomised, irradiated, baby mice was eventually superseded by the setting up of a colony of nude athymic mice. If such mice are irradiated, parasitised cells generally are not rejected.

A further significant advance in the use of mice for attenuating *T. parva* could be taken when it was found that the masses were not rejected by such mice. This advance was recently described by Irvin *et al.* (1975b). A steer was experimentally infected with ECF and, when it died 20 days later, lymph nodes were removed and a suspension of lymphoid cells prepared. Aliquots of the suspension were inoculated into irradiated athymic mice, which developed tumour-like masses 8–9 days later. The masses continued to grow without evidence of rejection until death of the mice, but there was no post mortem

evidence of parasite invasion of mouse cells, despite occasional observations in blood smears of piroplasms invading mouse erythrocytes. A mass from one mouse was removed post mortem, and a cell suspension prepared. Aliquots of this suspension were inoculated into further irradiated athymic mice, and in this manner, six passages were carried out. The behaviour of cells in mice was similar in all passages and no evidence of further adaptation of the parasite was observed.

In the most recent experiment in the series, Irvin *et al.* (1976) have shown that tumour-like masses produced in this way in mice can either be seeded into tissue culture or inoculated back into cattle. In a single experiment they showed that tumour material taken directly from mice, or passaged through tissue cultures, provoked mild reactions in recipient cattle, which were subsequently immune to lethal homologous challenge. If this can be repeated and developed, a mouse-attenuated vaccine strain of *T. parva* may be available for field trial.

Experiments have also been initiated by Irvin and his co-workers on the possible use of cell-fusion techniques to adapt *T. parva* to small animals. Irvin *et al.* (1974a) used tritiated thymidine to label *T. parva*-infected lympoid cells in culture, as was described by Moulton *et al.* (1971b). Larger amounts of tritiated thymidine of higher specific activity were used, and the label was taken up both by lymphoid cell nuclei and by the macroschizonts, as demonstrated by autoradiography. Aliquots of labelled culture were added to equal volumes of unlabelled *Theileria*-infected cultures, and it was then shown that multinucleate cells were formed and contained both labelled and unlabelled nuclei. This suggested that fusion had occurred, and also gave a possible explanation for the previously observed *in vivo* transfer of parasites from cells karyotyped as female (Malmquist and Brown, 1971).

Irvin *et al.* (1974c) then successfully fused *T. parva*-infected bovine lymphoid cells to baby hamster kidney (BHK) cells using Sendai virus, which had been inactivated with u.v. light as described by Harris *et al.* (1966). After the cultures had been grown in identical media, cell fusion was carried out in Leighton tubes with flying coverslips using standard techniques (Harris *et al.*, 1966). The resultant hamster/bovine heterokaryons were shown to be infected with *T. parva*. In some cases parasites were detected in cells which apparently contained only BHK nuclei. If selective growth can be achieved in subsequent experimentation, a non-bovine cell line parasitised with an attenuated form of *T. parva* may be obtained.

## VII. Cross-immunity

### A. Field trials

Cattle which recover from East Coast fever (ECF) are known to withstand homologous challenge, and for many years young stock were deliberately "salted" in East Africa by exposing them to challenge in an infected paddock. Animals which survived challenge were highly valued, particularly as draft oxen. In Wilde's review (1967) there is a clear indication that in his opinion and in the opinion of Barnett (1963) "all theilerial parasites that can be classified

as *T. parva* are cross-immunising". The classification tended to include *T. lawrencei*. It was with some confidence, therefore, that the team at EAVRO arranged for the Edinburgh University East African Veterinary Expedition of 1970 to go to Aitong in the Narok District of Kenya and compare the reactions of cattle immunised against *T. parva* (Muguga) with those of susceptible cattle. After all the cattle exposed at Aitong had died, this confidence was rather shaken!

Two experiments were carried out at Aitong as reported by Snodgrass *et al.* (1972). In the first, six susceptible cattle and 12 which had recovered naturally from infection and, in some cases, had also survived a lethal stabilate challenge, were exposed. All the animals died, having demonstrated fever, macroschizonts indistinguishable from *T. parva*, piroplasms, and anaemia. In the second experiment, eight susceptible cattle and eight cattle immunised against *T. parva* (Muguga) by recovery and subsequent challenge were used. Attempts were made to limit the ticks attaching to some of the animals by spraying with acaricide and also to treat some of the animals with primaquine diphosphate to eliminate theilerial piroplasms (Rogers and Callow, 1966). Once again, all the animals died. Tick numbers were reduced by spraying, and piroplasms eliminated by drug treatment.

The syndrome observed at Aitong was not typical of the disease induced in cattle by *T. parva* infection, in that high piroplasm parasitaemias and an associated anaemia were observed. Irvin *et al.* (1972d) carried out laboratory experiments to elucidate the nature of the ECF syndrome encountered. Blood from reacting cattle was transported on ice to EAVRO and inoculated into susceptible cattle. All the inoculated cattle became infected with a parasite which could be transmitted by inoculation to other cattle but could not be transmitted using *R. appendiculatus* ticks as vectors. In one instance ticks feeding on an animal receiving blood fresh from the field were able to transmit fatal ECF to a susceptible animal. Later studies have suggested that this may have been due to the mechanical transfer of theilerial macroschizonts circulating in the blood.

During the first experiment at Aitong, engorged nymphal ticks were collected from the ears of cattle reacting to the field challenge. These moulted in the laboratory and were found to be almost exclusively *R. evertsi*. They were either applied to cattle or attempts were made to harvest parasites from them for inoculation into susceptible cattle using the *in vitro* feeding technique (Purnell and Joyner, 1967) or grinding them (Purnell *et al.*, 1973a). None of the recipient cattle became infected. During the second experiment, *R. appendiculatus* nymphs from the laboratory colony were applied in ear-bags to the ears of reacting susceptible and *T. parva* (Muguga)-immune cattle. After moulting, these ticks were applied to susceptible and *T. parva* (Muguga)-immune cattle. The former animals died of typical ECF, although with abnormally high terminal piroplasm counts and some degree of jaundice evident on post mortem examination. The latter animals underwent mild ECF reaction. Attempts were made to transmit this tick-transmitted parasite by inoculation of parasitaemic blood from reacting animals, but no infections developed in the inoculated animals.

It appeared that at least two theilerial parasites were contributing to the ECF syndrome at Aitong. One was mechanically transmissible but not tick-transmissible. It appeared to be a pathogenic strain of *T. mutans*. The other was tick-transmissible but not mechanically transmissible. It appeared to be a strain of *T. parva* having some affinities with *T. parva* (Muguga). A third theilerial parasite may have been involved in the instance cited where ticks feeding on an animal receiving blood fresh from the field were able to transmit fatal ECF. In hindsight it now appears possible that this parasite was *T. lawrencei*, because subsequent investigations using material from the Aitong area (Stagg *et al.*, 1974) have shown that *T. lawrencei* macroschizonts may be readily harvested from the circulating blood of buffaloes.

Subsequent field trials were approached with more caution, and Cunningham *et al.* (1974b) described a successful trial in a *T. lawrencei* enzootic area of Kenya in which animals immunised against *T. parva* (Muguga) in combination with an apparently homologous strain of *T. lawrencei* survived while animals immunised against *T. parva* (Muguga) alone died as rapidly as susceptible animals. This result indicates clearly that the safest method of vaccinating cattle for exposure in a particular ECF area will be to obtain the local strain by feeding nymphal ticks on susceptible cattle exposed in a preliminary experiment, and then immunising cattle using this strain. This will also overcome local scepticism about the advisability of introducing alien strains of *Theileria* into an area, and the associated possibility of infecting local ticks which feed on vaccinated animals carrying low levels of alien piroplasms.

It should be emphasised that immunisation of cattle against ECF by infection with a "cocktail" of strains and chemoprophylaxis has been demonstrated to be effective against field strains of parasites picked up in areas widely removed from the point of origin of the constituent strains of the "cocktail" (Radley *et al.*, 1975d). Using a "cocktail" of two *T. parva* strains from central Kenya and one *T. lawrencei* strain from Northern Tanzania, Radley *et al.* (1975d) immunised cattle against strains of *Theileria* from central Uganda and the coast of Kenya. Further experimentation of titrating the infective inoculum of parasites and the dose of drug required to suppress the parasite multiplication beyond the desired immunogenic level, should result in the production of an infection and chemoprophylaxis vaccine which will be applicable throughout East Africa and will not induce a carrier status in immunised animals. Only if a number of small pilot field trials can then be carried out in various areas of East Africa will it be possible to ensure that a "cocktail" is developed which will protect against all the strains likely to be encountered. It was the strong recommendation of the WHO–FAO Joint Meeting of Investigators on the Immunology of East Coast fever, which took place in December 1974, that such field trials should take place as soon as possible. An East Coast fever vaccine is at hand, and it is to be hoped that the opportunity to develop it will be seized.

### B. WILD ANIMAL INVOLVEMENT

The role of the African buffalo (*Syncerus caffer*) in the epidemiology of East Coast fever syndromes in East Africa is well recognised. The two theilerial

parasites of the buffalo described by Brocklesby (1964) are both now thought to be pathogenic for cattle. *T. lawrencei* infections of cattle have been described by Brocklesby (1964) and also reviewed by Neitz (1957). *T. barnetti*, a new theilerial piroplasm of buffalo described by Brocklesby (1964), may well be synonymous with the pathogenic strain of *T. mutans* isolated at Aitong, since it originated from the same area and the two parasites have morphological similarities. Furthermore, Young *et al.* (1976a) transmitted a similar parasite from buffalo to cattle using *Amblyomma cohaerens* as the vector tick, and found that it was serologically identical to *T. mutans* (Aitong).

No other wild animal has been convincingly implicated in bovine ECF. Irvin *et al.* (1973) found theilerial piroplasms in blood smears from 34 of 87 impala (*Aepyceros melampus*) in Serengeti National Park, confirming the observations of Brocklesby and Vidler (1966). They also described a theilerial infection in the salivary glands of *Rhipicephalus evertsi* adult ticks fed as nymphs on some of the impala. Grootenhuis *et al.* (1976) also found theilerial piroplasms in blood smears from an impala and transmitted the parasite to another impala by blood passage. They failed, however, to transmit the parasite by this means to a splenectomised steer and also failed to transmit it to a steer by means of 500 nymphal *R. appendiculatus* ticks fed as larvae on the parasitaemic impala. Brocklesby and Vidler (1966) had similarly failed using a small number of *R. evertsi* adult ticks.

Brocklesby and Vidler (1961b) found theilerial piroplasms in blood smears from 50 of 58 wildebeest (*Connochaetes taurinus*) shot or captured near Aitong in the Narok District of Kenya. They also found a theilerial parasite in the salivary gland of an adult *R. appendiculatus* tick feeding on one of the wildebeest. They failed to transmit the wildebeest *Theileria*, named as *T. gorgonis*, using nine adult *R. evertsi* collected as engorged nymphs from a parasitaemic wildebeest to a steer. Purnell *et al.* (1973b) obtained two wildebeest from the Serengeti National Park and two from the Athi-Kapiti plains in Kenya. The wildebeest were splenectomised and their intra-erythrocytic theilerial parasitaemias exacerbated. In the case of the wildebeest from the Serengeti National Park they rose from 0 and 0·01% to 0·01% and 7% respectively, whilst the parasitaemias of the two Kenyan wildebeest rose from 0·01% to 72% and 82%. Blood from the two Kenyan wildebeest was inoculated into splenectomised cattle when the parasitaemias were 2·5%–44%, and none of the cattle became infected. Parasites were found in the salivary glands of four of 59 *R. appendiculatus* ticks fed as nymphs on one of the Serengeti wildebeest when its parasitaemia was 0·1%–30%, and in six of 58 *R. appendiculatus* ticks which dropped as engorged nymphs from one of the Kenyan wildebeest when its parasitaemia was 16%. A total of 476 adult ticks from the former wildebeest and 500 adult ticks from the latter were applied to steers, neither of which had a detectable reaction.

Brocklesby (1962a) described a piroplasm of the eland (*Taurotragus oryx pattersonianus*) from a single fatal infection of a yearling eland. Schizonts were discovered in sections of liver, lung and lymph node, and an intra-erythrocytic parasitaemia in excess of 90% was recorded. The parasite was named as *Cytauxzoon taurotragi*. Theilerial parasites were detected in the salivary glands

of both *R. appendiculatus* and *R. pulchellus* adult ticks collected as engorged nymphs from the eland. Transmission experiments were carried out, and two cattle, on each of which ten adult *R. appendiculatus* ticks were fed, failed to become infected. Another animal which received ten *R. pulchellus* ticks became febrile 6 days after tick application, and theilerial macroschizonts were demonstrable in lymph node biopsy smears from the 6th to the 9th day. Intra-erythrocytic piroplasms were found in blood smears from the 8th to the 12th day. It was subsequently immune to *T. parva* (Muguga) challenge. Irvin *et al.* (1972b) attempted to repeat this transmission of a piroplasm from eland to cattle. They fed large numbers of *R. appendiculatus* and *R. simus* nymphs on parasitaemic eland and demonstrated parasites in the salivary glands of the resultant adult ticks fed on eland from one area (Mount Kenya) but not from another (Galana Game Farm, Kenya). One hundred adult *R. appendiculatus* from Mount Kenya were fed on one steer, and 100 adult *R. simus* on another. Neither became infected. Brocklesby's positive results were an enigma until recently, when it was found that lymphoid cells from a parasitaemic eland could be grown in tissue culture to produce not one, but two, possibly distinct parasites (Stagg *et al.*, 1976). In four of five cell lines established from lymph node biopsy samples, theilerial schizonts were seen in lymphoblastoid cells, which grew in suspension. The fifth cell line grew as a monolayer, and the schizonts were seen to be infecting monocytic macrophage-like cells. Many infected multinucleate cells were seen in this culture, and the multiple schizonts resembled the cytomeres of *Cytauxzoon taurotragi* described by Brocklesby (1962a). It is possible that the parasite in the four cell lines may have been a species of *Theileria* and that in the fifth cell line may have been *Cytauxzoon taurotragi.* If the *Theileria* later proves to be infective for cattle, Brocklesby's results will be explicable, and the long-standing belief that implicates the eland in the ECF syndrome will gain substance.

There are various other wild members of the family Bovidae in East Africa whose habitat results in them attracting large numbers of rhipicephalid ticks, and they are known to harbour intra-erythrocytic theilerial piroplasms (Brocklesby and Vidler, 1965). It remains to be seen whether any of them are capable of harbouring theilerial pathogens of cattle.

## VIII. Immunodiagnosis

One of the major advances in East Coast fever research in recent years has been the development of a reliable serological test. By this means, the diversity of strains of *Theileria* has been realised and experimental results have been interpreted with greater confidence.

Several different tests have been developed. The complement-fixation (CF) test and the indirect fluorescent antibody (IFA) test were first applied to ECF by Schindler and Wokatsch (1965), the capillary-tube agglutination (CA) test by Ross and Lohr (1972), and the indirect haemagglutination (IHA) test by Duffus and Wagner (1974). While all these tests have their adherents, there is no doubt that the IFA test has been the most used and useful.

Lohr and Ross (1969) used the IFA test to detect antibodies against *T. parva*

in bovine sera. Negative sera were obtained from cattle either reared in tick-proof, disease-free, isolation or recently imported into Africa from Northern Europe. Control positive sera were obtained from cattle recently recovered from experimental *T. parva* infection. The two different antigens used were both obtained from calves experimentally infected with *T. parva.* Films of highly parasitaemic blood were used as piroplasm antigens, and impression smears from the spleens of animals removed at post mortem were used as schizont antigens. Using a standard IFA technique, Lohr and Ross demonstrated negative reactions with negative sera and high positive ones with control positive sera. Piroplasm antigen was shown to give more consistent results. Cross-reactions occurred with sera from *T. lawrencei*-infected cattle, and, to a limited extent, with sera from cattle infected with blood-passaged *T. mutans.* Using homologous and heterologous antigens, they were able to titrate differentially antisera from single experimental infections with *T. parva* and *T. mutans.* The test was used to measure primary antibody response in nine cattle reacting to and recovering from experimental *T. parva* infection. Antibodies were first detected 8 to 14 days after initial exposure, rising to a peak by about the 30th day and then declining gradually but remaining detectable for at least 12 months.

Burridge (1971) applied the IFA test to the study of experimentally-induced ECF, using washed infected erythrocytes as a piroplasm antigen. Negative control sera were obtained from splenectomised calves reared in England. The positive control serum was collected from a recovered steer 4 weeks after experimental infection with *T. parva.* Three categories of serum sample were tested: (a) pre-infection sera from 115 cattle, (b) post-infection sera from 45 cattle recovered from patent infection with *T. parva,* (c) post-infection sera from 70 cattle that showed no patent reaction to experimental infection with *T. parva.* Many of the pre-infection sera showed titres of 1 in 160, which was used as the baseline for sera in the other two categories. Of the 45 cattle recovered from patent infection, 44 showed positive titres and were subsequently immune to lethal stabilate challenge, whilst one did not have a positive titre and died on challenge. Of the 70 cattle with no patent reaction to infection, 61 had no positive titre and were susceptible on challenge whereas 9 had positive titre and only one was susceptible on challenge. This showed that the IFA test was a more accurate method of assessing experimentally-induced *T. parva* immunity than was the standard technique of examination of Giemsa-stained lymph node biopsy smears.

Kimber and Burridge (1972) described a modification of Burridge's techniques, by which the collection of blood from test animals could be simplified. The test could then be used for routine serological screening of cattle and in epizootiological studies on East Coast fever. Blood was collected from 95 test animals and allowed to air-dry as blood spots on filter paper. At the same time serum was prepared from each animal by the standard method. Eluates of the dried blood were made by allowing the filter papers to soak in phosphate-buffered saline (PBS), pH 7·0 for 2 h at room temperature. The amount of blood and PBS could be adjusted so that dilutions of blood up to 1:5000 were obtained. Titration of the conventionally obtained sera and of the dilutions

obtained from dried blood showed that there was a good correlation in the results obtained by the two methods. The baseline for dried blood dilutions was 1:50 and the peak titre 1:5000, compared to 1:160 and 1:40 960 for the conventional dilutions. A study of the antibody response in a sublethally infected cow using the two methods also demonstrated the correlation between them, although the conventional method was felt to be a more accurate quantitative assessment of the response.

The development of a method of growing *T. parva* macroschizonts in tissue culture (Malmquist *et al.*, 1970) meant that a new source of antigen was available for the IFA test. The tissue culture material contained no particulate matter except lymphoid cells, of which at least 80% were parasitised with macroschizonts. The material was easily prepared in large volumes and could be standardised by using the same infected cell line for each batch of antigen. Burridge and Kimber (1972c) compared schizont and piroplasm antigens using the sera of 59 cattle recovered from experimentally induced ECF. Antibodies to schizont antigen were first detected 3 weeks after infection and to piroplasm antigen 1 week later. The peak titres to both antigens were rapidly reached, in most cases a week after the initial antibody rise, and were generally higher to piroplasm antigen. Eight cattle were tested for 26 weeks after infection, and significant titres were detected to schizont antigen for about 21 weeks but to piroplasm antigen for only about 6 weeks. Six calves infected with *T. mutans* were tested using both antigens, and slight rises in titre were observed using piroplasm antigen whereas none was observed using schizont antigen. The authors concluded that cell culture schizont antigen is to be preferred to piroplasm antigen because of its greater specificity and reliability and because of the ease with which its preparation could be standardised. They then used schizonts as antigen and dried blood samples as the source of serum (Burridge and Kimber, 1972b) and showed that they could be used together in the IFA test to give accurate results. In further studies (Burridge and Kimber, 1973) the authors investigated the duration of serological response to the IFA test of cattle recovered from *T. parva* infection using schizont antigen. They demonstrated the long-term detection of antibodies using this system and suggested that it should be used in sero-epizootiological studies of ECF.

Schindler *et al.* (1969) used *T. parva* piroplasm antigen in the IFA test to detect antibodies in the sera of cattle recovering from a Rhodesian strain of *T. lawrencei,* but their interpretation of the relationship between the two theilerial species was hampered by their lack of *T. lawrencei*-infected material for antigen preparation. In their serological studies of *T. lawrencei* infections in cattle, Burridge *et al.* (1973) were able to use *T. lawrencei* piroplasms from a strain of the parasite transformed by cattle passage so that it produced high piroplasm parasitaemias (Young and Purnell, 1973b) and *T. lawrencei* schizonts grown in tissue culture using techniques similar to those described by Malmquist *et al.* (1970). An experiment was carried out using seven cattle recovered from *T. lawrencei* infection and eight cattle recovered from *T. parva* infection. A *T. lawrencei* piroplasm antigen was prepared from an animal with a 17% parasitaemia, and *T. lawrencei* schizont antigen from an *in vitro* culture suspension of lymphoid cells, 90% of which were infected with *T.*

*lawrencei* macroschizonts. Antigens of *T. parva* piroplasms and schizonts and of *T. mutans* piroplasms were also used.

The serological responses of the 15 cattle to these various antigens could then be studied. Sera from the seven cattle recovered from *T. lawrencei* infection had significant increases in titre to both *T. lawrencei* and *T. parva* schizont antigen and all but one had increased titres to *T. lawrencei* and *T. parva* piroplasm antigen. The titre levels obtained generally corresponded where schizont or piroplasm antigens of the two theilerial species were used. The results indicated that *T. lawrencei* and *T. parva* are antigenically indistinguishable in the IFA test and thus demonstrate the close antigenic relationship between the two species.

After the development of the technique for growing buffalo lymphoid cells infected with *T. lawrencei* in cell suspension (Stagg *et al.*, 1974), their use as an antigen in the IFA test could be investigated. Bovine antisera to *T. parva* and *T. lawrencei* and buffalo antisera to *T. lawrencei* were used by Burridge *et al.* (1974a), and complete cross-immunity between buffalo *T. lawrencei* schizont antigen and bovine *T. lawrencei* and *T. parva* schizont antigen were demonstrated. This result indicated once more that the IFA test could not be used to differentiate between the two *Theileria* species.

The refinement of the IFA test for *Theileria* which has been carried out by Burridge and his co-workers has resulted in a clearer interpretation of the antigenic relations of theilerial parasites freshly isolated from the field. The ECF syndrome at Aitong, described by Irvin *et al.* (1972d), was examined from the serological viewpoint by Burridge and Kimber (1972a). The sera from the cattle infected with either the tick-transmitted or the mechanically transmitted parasite were examined using *T. parva* (Muguga) schizont and piroplasm antigens, *T. mutans* piroplasm antigen (whose origins were described by Lohr and Ross, 1969), and piroplasm antigens derived from cattle reacting to the two separated homologous strains.

Of the 18 animals infected with the mechanically transmitted parasite, one died within 3 weeks of infection and showed no raised antibody titres. The remaining 17 animals all showed significant responses to both *T. mutans* and the homologous mechanically transmitted piroplasm antigen. The correlation between these responses suggested that this parasite was a strain of *T. mutans* and it was designated *T. mutans* (Aitong).

Ticks infected with the tick-transmitted strain of *Theileria* from Aitong were applied to two *T. parva* (Muguga)-immune cattle. Both animals reacted to the parasites introduced by the ticks, and they showed a marked rise in antibody titre to *T. parva* (Muguga) and to the homologous tick-transmitted piroplasm antigens following recovery. They showed no response to the other piroplasm antigens. The pattern of antibody response to the two *T. parva* antigens was similar, showing that the tick-transmitted parasite behaved antigenically like typical *T. parva* in the IFA test. It was designated *T. parva* (Aitong).

An unusual strain of *Theileria* was isolated by Burridge *et al.* (1974b) from the Kiambu District of Kenya both by feeding *R. appendiculatus* adults collected in the field on susceptible animals in the laboratory and by feeding laboratory reared *R. appendiculatus* nymphs on reacting animals from the

field. Reactions of infected cattle were characterised by the production of low numbers of macroschizonts, no microschizonts, few or no piroplasms, and a transient febrile response. All the cattle recovered from infection and were fully susceptible to subsequent *T. parva* (Muguga) challenge. A cell culture isolate of *Theileria* sp. (Githunguri) was made from infected lymphoid cells of a reacting animal, and from the resultant *in vitro* culture suspension, a schizont antigen of the parasite was prepared. The sera of the cattle recovered from the infection and the schizont antigen were tested in an IFA matrix against *T. parva* (Muguga) and its schizont antigen; *T. lawrencei* (Serengeti) and its schizont antigen; and *T. mutans* (Aitong) and its piroplasm antigen. The results indicated that *Theileria* sp. (Githunguri) was antigenically distinct from *T. parva*, *T. lawrencei* and *T. mutans* but had some antigens in common with *T. parva* and *T. lawrencei*. Its specific identity is thus unknown and it is possibly a *Theileria* originating from wild game which frequent the farm from which it was isolated.

## Acknowledgements

This review was carried out with the full and willing co-operation of the staff of the UNDP/FAO Immunological Research on Tick-borne Cattle Diseases and Tick Control Project, of which I have recently been the Project Co-manager. I am particularly indebted to Mr M. P. Cunningham, the Project manager, Mr C. G. D. Brown, Dr A. S. Young and Dr D. E. Radley for their help in its preparation. Dr D. W. Brocklesby, Head of the Parasitology Department at IRAD, and Mr A. D. Irvin have been most helpful in their advice on the manuscript. Finally, I am grateful to H.M. Government Overseas Development Ministry for employing me in Africa; and to Dr G. L. Corry, the Director of the East African Veterinary Research Organization, and Dr J. M. Payne, the Director of IRAD, for providing me with facilities to write this review.

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# Seasonal Occurrence of Helminths in Freshwater Fishes
# Part I. Monogenea*

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## I. Introduction

The earth is endlessly engaged in a variety of motions. The most obvious is the earth's daily rotation on its own axis, leading to the alternation of day and night and the phenomenon of the circadian rhythm in living matter. A longer term

* This review will be completed by Part II to appear in "Advances in Parasitology" Vol. 17.

motion, that of the earth revolving around the sun in almost exactly 365¼ days, leads to annual phenomena and is responsible for the succession of the seasons. It is the tilt of the earth's axis which initiates the seasons, as it is the angle at which the rays from the sun strike the earth, rather than the distance they travel from the sun, that is the critical factor in determining the earth's seasonal changes in temperature. A shaft of sunlight that strikes a surface at right angles provides twice as much energy per square metre as does a shaft of sunlight striking it at an angle of 30°. It is summer in either hemisphere when the rays of the sun come closest to striking the surface at a right angle. It is winter when they are the most oblique.

Seasonal changes in nature are very clearly reflected in organic life. It is therefore natural to expect that parasites, ectoparasites especially, but also endoparasites although they have no direct ties with the external environment, should not remain indifferent to the annual cycle of climatic changes. Indeed, seasonal changes in the incidence of some species of parasites are not infrequent and have often been recorded in the literature (Dogiel, 1964). Hawking (1975) reviewed the occurrence of circadian and annual rhythms of parasites, but his account does not include more than a mention of fish parasites. Indeed, it is perhaps surprising that there is no comprehensive review of seasonal occurrence of fish parasites. This first part of my own review is concerned with the seasonal occurrence of monogeneans of freshwater fishes.

The current phylogenetic concept is to consider monogeneans as one of five classes within the Phylum Platyhelminthes (Hargis, 1971a). The members of the Class Monogenoidea are ectoparasites of the gills, skin and orifices of fishes, and, less frequently, of the oesophageal tracts and bladders of amphibians and turtles (Hargis, 1971b).

The monogeneans have a direct life-cycle with a single host, although Bykhovskii and Nagibina (1967) found evidence of "intermediate" hosts in the life-cycles of *Pricea* and *Gotocotyla*. Apart from the viviparous Gyrodactylidae, where the newly born young resemble their parents, an egg is laid. The egg hatches to give a ciliated larva (oncomiracidium). This infects the host, leading to a non-ciliated juvenile which grows to give the adult worm. The larval development of monogeneans has been summarised by Bykhovskii (1957) and Llewellyn (1963, 1968). Details of the invasion of the fish host are also provided by these authors. The life-cycles were divided into three major types by both Bykhovskii and Llewellyn. The simplest (Dactylogyridae, Tetraonchidae) have a prolonged period of egg deposition into the water, with free-swimming larvae capable of infecting the host at all stages of its development. Growth of these monogeneans from egg to adult is rapid; for instance, with *Dactylogyrus vastator* at 20°C, the life-cycle was completed in 10–12 days (Kollmann, 1972). This form of life-cycle is found in the genus *Dactylogyrus*, although some species, as *D. iwanowi*, have a more complex cycle because the host is a migratory species of fish.

Some life-cycles, such as those of *Diplozoon paradoxum* and *Discocotyle sagittata* (Discocotylidae), show a shortening of the period of egg production, a concentration of egg deposition onto or near the potential hosts, and an increased adaptation of the parasite life-cycle to the life-cycle of its host.

These larger monogeneans have a slower development before reaching sexual maturity and a life span of one or more years.

The viviparous Gyrodactylidae have rapid development and a short life span. Specimens of *Gyrodactylus alexanderi* maintained at 15°C on isolated fish, gave birth to two daughters, the first after an average of 1·6 days, and the second after 6·9 days. Maximum longevity at 15°C was 28 days, although longer at lower temperatures (Lester and Adams, 1974b).

As Kennedy (1975) has indicated, it is important to distinguish incidence from maturation cycles, since there is no consistent correlation between them. Although all species which exhibit seasonal incidence and growth cycles also show seasonal maturation, species which are not seasonal in their incidence do not always breed throughout the year.

Parasitologists normally consider the following features of each fish–parasite relationship. The incidence of the parasite, expressed as percentage infection, is established for the sample of fishes, which should be representative of the population under study. Ideally, the fishes should be divided into length groups or age classes, and the percentage infection given separately for each, as infection usually varies during the life of a fish according to changes in its size and behaviour. The intensity of infection is determined by counting the numbers of parasites found on or in each organ system. The maturation of the parasites is assessed by careful study of all stages in the first instance; thereafter it may be possible to divide what is normally a continuous process into a number of meaningful and relatively discrete stages which can be used to delimit the state of development towards ultimate maturity.

The incidence of infection may be seasonal or throughout the year. Within the time of occurrence the incidence may remain more or less constant, or change in a regular or an irregular manner.

The intensity clearly follows incidence. Within the incidence pattern of a species the intensity may remain more or less constant, or change in a regular or an irregular manner.

The maturation of parasites also follows incidence. Within the incidence pattern of a species the maturation may remain more or less constant, or change in a regular or an irregular manner.

Where incidence, intensity or maturation processes appear to be more or less constant, as shown by numerical data, a dynamic equilibrium is likely to exist, where for incidence and intensity the gain of a parasite is balanced by a loss of a parasite, or for maturation where the sexual cycle is repeated continuously. For instance Chubb (1963) observed such a dynamic equilibrium for the cestode *Triaenophorus nodulosus* in the intestine of *Esox lucius*.

Studies on the monogeneans have shown that the patterns of incidence, intensity and maturation may vary in relation to the age of the host. As such studies have normally avoided any seasonal bias, they are omitted here, except where they have been shown to have relevance to seasonal investigations.

In this review Section II reports the studies on seasonal occurrence. Whilst this section aims to be comprehensive, some relevant material has necessarily been omitted, either through lack of knowledge of the work by the author, or because the literature had not become available at the time this review was

written. In Section II the aim has been to include the factual information in summary, but no attempt has been made to interpret each and every observation. Section III, using the observations from Section II, shows where seasonal studies on monogeneans have been carried out on a world-wide level, related to the major climatic zones. Section IV attempts to draw the current knowledge together into meaningful conclusions and to suggest the manner in which further data could be collected to assist in our understanding of the seasonal dynamics of the monogeneans.

## II. Seasonal Studies

Seasonal occurrence is reported under families following the classification of Bykhovskii (1957). Within families, alphabetical order is adopted.

### A. SUBCLASS POLYONCHOINEA

#### 1. *Order Dactylogyridea*

#### (a) *Family Dactylogyridae*

*Actinocleidus fergusoni* Mizelle, 1938

Studied by Crane and Mizelle (1968) in Sacramento County, California, U.S.A., this species was present on *Lepomis macrochirus* during all months of the year. Developmental stages were observed throughout the investigation, showing that the life-cycle proceeded continuously in the temperature conditions of California. Population peaks showed no consistent relationship with temperature. Rawson and Rogers (1972b), working on *L. macrochirus* at the Walter F. George Reservoir, Alabama, U.S.A., also found *A. fergusoni* during all months of the year. Its population increased from a December low to a moderate population in late February, then declined during spring. In June it increased, to a high level at the warmest July temperatures, and during the remaining summer and autumn months the population was at a moderate level, but by December it had risen to the highest level observed. Rawson and Rogers (1972b) placed this species in their Group 3, small species abundant during the summer but with a prewinter peak at water temperatures near 10°C.

*Actinocleidus fusiformis* (Mueller, 1934)

Rawson and Rogers (1972a) found this species on *Micropterus salmoides* in the Walter F. George Reservoir in Alabama, U.S.A. throughout the year. Abundance increased during the winter months, reaching the highest level in mid-March. During the spring period of maximum temperature change abundance decreased, but then expanded to a peak in late June. Abundance was maintained at a moderate level during the summer, with a mid-September peak. In the autumn the abundance declined, but rose again during the autumn maximum temperature change in late October to its highest level. During November and December a slow fall in numbers occurred.

*Anchoradiscus triangularis* (Summers, 1937)

Studied in the Walter F. George Reservoir, Alabama, U.S.A. on *Lepomis macrochirus* by Rawson and Rogers (1972b), this species belonged to their

Group 1, large species most abundant during autumn and least abundant during the mid-summer period. The population was negatively related to high water temperature. The parasite was at moderate levels of occurrence initially in December and January, and it decreased gradually to its lowest level during July and August. The population fluctuated but increased gradually during the autumn to a peak in early December, then fell again to moderate levels.

*Ancyrocephalus paradoxus* Creplin, 1839

Parasitising *Perca fluviatilis* in the Rybinsk Reservoir, U.S.S.R., this species was reported by Izyumova (1958b) all year round, but with a lowered rate and intensity of infection during winter. Wierzbicki (1970) found *A. paradoxus* only in the autumn and January in Lake Dargin (Mamry), Poland. Wegener (1909) and Malakhova (1961) had similar findings, and Malakhova stressed the pronounced seasonal occurrence of this species. In the light of the errors in the descriptions of some species noted by Bykhovskii (1957), the revision of Czechoslovak material by Ergens (1966), with the description of *Ancyrocephalus percae* n. sp. in the latter paper, and the revision of the genus by Bykhovskii and Nagibina (1970), it is likely that the conflicting patterns of occurrence can be explained by a confusion of specific identity.

Komarova (1964) found *Lucioperca lucioperca* in the Dnepr Delta, U.S.S.R., to be infected during all months she examined these fish, i.e. April to August, and October. The percentage and intensity of infection did not vary a great deal, but both were highest during June. Fish were not examined during the remaining months.

*Ancyrocephalus percae* Ergens, 1966

Rautskis (1970a) examined *Perca fluviatilis* from Lake Dusia, Lithuanian S.S.R., during all the year, and found a 6·6% infection in the January to March period.

*Clavunculus bifurcatus* (Mizelle, 1941)

Studied by Rawson and Rogers (1972b) on *Lepomis macrochirus* in the Walter F. George Reservoir, Alabama, U.S.A., this was one of their Group 1 species, most abundant during autumn and least abundant during the mid-summer period. The population decreased from an initially low level in December and January and was then rarely detected until October. During the autumn the population increased steadily to a peak in early December, then fell to the level of the previous winter.

*Clavunculus bursatus* (Mueller, 1936)

Rawson and Rogers (1972a) investigated this species on *Micropterus salmoides* in the Walter F. George Reservoir, Alabama, U.S.A. After the initial December sample, abundance declined, but gradually increased to a peak in early February at a water temperature of 9°C. Abundance was at a low level in late March and early April and again in late September and October, during the periods of rapid temperature change, but in the remaining spring and summer months it varied at a moderate level. In December the abundance again reached a peak at a water temperature of 9°C.

*Cleidodiscus nematocirrus* Mueller, 1937

Studied by Rawson and Rogers (1972b) in the Walter F. George Reservoir, Alabama, U.S.A., on *Lepomis macrochirus*. Belonging to their Group 2, large species most abundant during the spring, it was rare during most of the investigation but did reach its maximum level during the warmest summer temperatures.

*Cleidodiscus robustus* Mueller, 1934

Also investigated by Rawson and Rogers (1972b) in the Walter F. George Reservoir, Alabama, U.S.A., on *Lepomis macrochirus*. A large species most abundant in spring, it first appeared in March. The population rose to a peak in June, then disappeared from the samples.

*Dactylogyrus alatus* Linstow, 1878

Found by Izyumova (1964) on *Blicca bjoerkna* at the Rybinsk Reservoir, U.S.S.R., predominately in spring and summer.

*Dactylogyrus amphibothrium* Wagener, 1857

Wootten (1974) studied this species on *Gymnocephalus cernua* in Hanningfield Reservoir, Essex, England. Wootten (pers. comm.) found that *D. amphibothrium* occurred all year on the fish; the incidence of infection was highest from March to July, although this was almost certainly a reflection of the fact that higher numbers of large fish were examined at this period. Eggs were present in the worms from February to August. Izyumova (1964) found this species all year at the Rybinsk Reservoir, U.S.S.R.

*Dactylogyrus anchoratus* (Dujardin, 1845)

Studied in detail by Prost (1963) on *Cyprinus carpio* at a fish farm in Opole Lubelskie, Poland. Adult worms could overwinter on the fish, and the infection increased rapidly during spring and summer. A decrease in numbers of adults on the fish occurred in November in Poland. Oviposition started in early spring, and the intensity increased as the temperature rose to an optimum about 23°C. Below 5°C oviposition ceased for the winter. Eggs laid in the autumn overwintered on the bottom of the pond if covered by water and some hatched during spring as water temperatures rose. The larva in the egg developed to hatching in $1\frac{1}{2}$ days at 28°C–29°C, 8 to 9 days at 16°C, and no development occurred at 3°C. Fry were infected from their 13th day of life. The larva developed to an adult to commence oviposition in 6 days at 23°C–25°C and in 12 days at 16°C. Adult worms could live at least 42 days on the fish at 20°C–23°C. Gröben (1940) also provided details about the development of *D. anchoratus*.

*Dactylogyrus aristichthys* Long & Yu, 1958

Musselius (1968) studied this species found on *Aristichthys nobilis* in the U.S.S.R. It reproduced at temperatures from 14°C–15°C to 28°C–30°C (Bauer *et al.*, 1969). In the Moscow area reproduction began in mid-May and continued until late August, while in the more southern Krasnodar Territory it was from late April to September. Reproduction was most intense at water temperatures of 22°C–25°C. The eggs developed attached to objects in the water. The duration

of embryonic development was determined by water temperature: hatching occurred after 2 days at 30°C, 3½ days at 21°C–22·6°C, 4 days at 17°C–19°C, 10 days at 12°C and 15–16 days at 5°C–8°C. Freezing killed the larvae. Development from egg to maturity lasted 11–13 days at a water temperature of 17°C–23°C.

*Dactylogyrus auriculatus* (Nordmann, 1832)

Komarova (1964) found *Abramis brama* in the Dnepr Delta, U.S.S.R., to be infected in February, April, May and June, but not in March, July–August and October. Incidence and intensity during months when infections were found varied between 13·2% (May) to 40·0% (April) and 16 to 43 worms per fish. Wierzbicka (1974) found *Abramis brama* in Lake Dabie, near Szczecin, Poland, to have a general incidence of 65·8%. Worms were found most often in the spring and summer months (72·7%–100%) and from September to November the incidence dropped considerably (9·1%–40%). Related seasonal fluctuations of intensity of infection were also clearly visible. Izyumova (1964), at the Rybinsk Reservoir, U.S.S.R., also observed *D. auriculatus* predominantly during spring and summer.

*Dactylogyrus borealis* Nybelin, 1936

Studied by Molnár (1968) in Lake Balaton, Hungary, on *Phoxinus phoxinus*, together with *D. merus* and *D. phoxini*. Found in April, June, July and October, but not during the other months.

*Dactylogyrus cabolleroi* Prost, 1960

Kashkovski (1967) found *Rutilus rutilus* at the Iriklin Reservoir, River Ural, U.S.S.R., to be infected from April to August. Worms were not found September to March. Maximum incidence was in June, maximum intensity in April. Strelkov *et al.* (1969) noted that in Vrevo Lake, near Leningrad, U.S.S.R., *Rutilus rutilus* were rather seldom infected by *D. cabolleroi*, hence the seasonal dynamics had not been studied there.

*Dactylogyrus chranilowi* Bykhovskii, 1933

Studied by Wierzbicka (1974) on *Abramis ballerus* in Lake Dabie, near Szczecin, Poland. The incidence of infection was very high: in the spring, summer and some autumn samples it was 100%. A lowered incidence (70·6%–84·6%) was recorded in some autumn and winter months, and the intensity of infection was also lower in the autumn and winter. It parasitised *A. ballerus* all year at the Rybinsk Reservoir, U.S.S.R. (Izyumova 1964).

*Dactylogyrus cordus* Nybelin, 1936

Davies (1967) recorded *D. cordus* on *Leuciscus leuciscus* in the River Lugg, Herefordshire, England. The worms were not found from October to December. Incidence increased from January, reaching a peak in April (63%). No worms were found in May. Worms occurred again in June, with a second peak in July (63%). The incidence declined during August and September.

*Dactylogyrus cornoides* Gläser & Gussev, 1967

Wierzbicka (1974) found *D. cornoides* on *Blicca bjoerkna* in Lake Dabie, near Szczecin, Poland. 57·6% of the fish were infected; the peak of infection was in the spring and summer months and the lowest incidence in the late autumn and winter seasons.

*Dactylogyrus cornu* Linstow, 1878

Studied by Komarova (1964) at the Dnepr Delta, U.S.S.R., on *Blicca bjoerkna* and *Vimba vimba vimba* natio *carinata*. On *B. bjoerkna* incidence increased from February to a maximum (100%) in May and June. A sample in October showed the incidence to be 80%. Intensity of infection was at a maximum in June. On *V. v. vimba* natio *carinata* the February and April–May incidences were highest, it was not found in March, and the June–July incidence was fairly low (33·3%), although maximum intensity occurred during these months. Wierzbicka (1974), on *B. bjoerkna* in Lake Dabie, near Szczecin, Poland, found maximum incidence, up to 100%, in the spring and summer months, the incidence being lowest in the autumn and winter months (11·1%–25%). The intensity of infection followed a similar seasonal pattern. Kashkovski (1967) found *D. cornu* on *Rutilus rutilus* in the Iriklin Reservoir, River Ural, U.S.S.R., in April and September only. *Rutilus rutilus* is an occasional host for *D. cornu*, and the incidence during both months was low (6·6%). Izyumova (1960) found *D. cornu* on *Blicca bjoerkna* during the summer only at the Rybinsk Reservoir, U.S.S.R.

*Dactylogyrus crucifer* Wagener, 1857

Recorded by Komarova (1964) at the Dnepr Delta, U.S.S.R., on *Rutilus rutilus heckeli* during all months investigated, February to October, excluding September. Incidence and intensity were high throughout (73·3%–100%, 8–156 worms), but incidence was 100% during April to June. Kashkovski (1967), on *Rutilus rutilus* in the Iriklin Reservoir, River Ural, U.S.S.R., found maximum incidence (100%) during May to July. No worms were found in January, and incidence increased during February to May. After July, incidence fell during August to November, and no worms occurred in December. Intensity was highest in June. In England, Rizvi (1964, 1969) investigated *R. rutilus* at Rostherne Mere, Cheshire. No worms were found from September to January. Incidence was maximum (90%) in May, after increasing during February to April, and remained high during June and July. Incidence fell in August, to nothing in September. Mishra (1966) worked on *R. rutilus* in the Shropshire Union Canal, Cheshire. He found worms with eggs during all months of the year. Minimum incidence was in December (12%). It increased during January to April (81%), fell a little in May (72%) and remained high during June (81%) to September (93%). Maximum incidence was 95% in July. During October and November it fell to the December minimum. Davies (1967) examined *R. rutilus* from the River Lugg, Herefordshire, and found a pattern of occurrence more similar to that of Rizvi than Mishra. She found no *D. crucifer* during January. Incidence increased during February and March to a maximum in April (100%). In May incidence fell markedly to 11%, but it increased to 77% in June and 96% in July. In August it decreased to 67% and during September to December no worms were found. It is interesting to note these variations in seasonal occurrence within a relatively small geographic area, but no explanation can be offered as the three investigations were carried out during different years. Rumyantsev (1972) studied *D. crucifer* on *R. rutilus* in Lake Srednego Kuĭto, Karelia, U.S.S.R. He found that the lower the water temperature in

May and June, the greater the displacement of the incidence peak. In May and June 1962 the water temperature did not increase above 10°C until after the first third of June and *D. crucifer* was not found until July. In 1963 the water temperature was above 10°C from the first third of May and *D. crucifer* was found in May. Variation in annual temperature patterns have considerable significance in relation to patterns of occurrence of the smaller monogeneans.

*Dactylogyrus ctenopharyngodonis* Akhmerov, 1952

According to Bauer *et al.* (1969) the development of this species on *Ctenopharyngodon idella* is unknown, but it was most numerous in midsummer at a high water temperature.

*Dactylogyrus distinguendus* Nybelin, 1936

Wierzbicka (1974) studied this species on *Blicca bjoerkna* at Lake Dabie, near Szczecin, Poland. Despite considerable fluctuations in incidence in single months, a certain correlation with the seasons was found. It was not found in November and December, and had maximum incidence during the summer months.

*Dactylogyrus extensus* Mueller & Van Cleave, 1932

Infecting *Cyprinus carpio*, temperature has been shown to be an important factor in the reproduction of this species (Bauer and Nikol'skaya, 1954; Ivasik, 1953; Prost, 1963). It occurred, and reproduced, even at the coldest period of the year (Bauer, 1959a). At 3°C development of the larva in the egg was inhibited, but at least some remained viable to hatch when the temperature was raised (Prost, 1963). At 16°C–17°C, 70%–80% of the eggs laid produced larvae, but at 22°C–26°C only 20%–30% hatched. According to Bauer (1959a) at 20°C only 9% hatched. The optimum temperature for the species was 16°C–17°C (Prost, 1963). Ivasik (1953) found a decrease in parasite numbers during the summer months of high temperature in Ukranian fish farms.

Paperna (1964a), contrary to the above data, found that *D. extensus* thrived at the normal summer temperature (24°C–28°C) in Israel. The worms were identical in morphology with those from northern Europe and North America. *C. carpio* were first introduced into Israel from eastern Europe about 1920, and the *D. extensus* must be descended from imported individuals which have become adapted to the higher summer temperatures normal in Israel. Izyumova and Zelentsov (1969), however, experimentally infected young *C. carpio* with *D. extensus* larvae and investigated the life-cycle of the worms at temperatures of 14°C–15°C and 17°C–19°C. The full life-cycle required 18–20 days at both temperature ranges. They suggested that *D. extensus* was well adapted to grow and reproduce at both low and high temperatures.

*Dactylogyrus falcatus* (Wedl, 1857)

Bogdanova (1958) found this species on *Abramis brama* in the River Volga, U.S.S.R., during the spring and summer. Izyumova (1964) reported a similar incidence on the same host at the Rybinsk Reservoir, U.S.S.R. Komarova (1964), in the Dnepr Delta, saw no worms on *A. brama* in February, June or October, but a 21·3%–33·3% infection in March to May and July and August. Maximum intensity was in August. Kashkovski (1967) recorded 6·6% of

*Rutilus rutilus* infected by *D. falcatus* in May, but no other month, at the Iriklin Reservoir, River Ural, U.S.S.R. *R. rutilus* is an accidental host for *D. falcatus*. At Lake Dabie, near Szczecin, Poland, Wierzbicka (1974) observed *A. brama* to have a general incidence of infection of 63·9%. The incidence increased slightly in spring, but there were no marked seasonal changes of incidence or intensity of infection.

*Dactylogyrus fallax* Wagener, 1857

Observed by Izyumova (1964) at the Rybinsk Reservoir, U.S.S.R., predominantly in spring and summer, usually disappearing during the winter. Kashkovski (1967) found *Rutilus rutilus* in the Iriklin Reservoir, River Ural, U.S.S.R., to be infected at low level (6·6%) in February, and at higher levels (46% and 26% respectively) in May and June. No worms were found during the other months. Rumyantsev (1972) reported on the effect of annual differences in water temperatures on *D. fallax*, in Lake Srednego Kuito, Karelia, U.S.S.R. The low May and June temperatures in 1962 prevented occurrence until July, but in 1963, when the water temperatures in May and June were higher, the worms occurred during these months, but had disappeared by July. In Lake Dabie, near Szczecin, Poland, Wierzbicka (1974) found *D. fallax* as the rarest monogenean parasite of *Blicca bjoerkna*. It had a remarkable seasonal occurrence, being almost exclusively found in spring, when the incidence reached a peak of 75% by March and the worms disappeared by June. 16·7% of the fish were infected in December but no fish were caught during January and February.

*Dactylogyrus haplogonus* Bykhovskii, 1933

*Vimba vimba vimba* natio *carinata* were examined by Komarova (1964) in the Dnepr Delta, U.S.S.R., from February to July and in October. *D. haplogonus* was found in March to May, with peak incidence 26·6% in March.

*Dactylogyrus hypophthalmichthys* Akhmerov, 1952

Occurred on *Hypophthalmichthys molitrix* from April to October (Bauer *et al.*, 1969). In the Krasnodar Territory, U.S.S.R., fish up to yearlings had an incidence of 100%, with an average intensity of 6·3–12·5 worms each. Three- to five-year-old fish may each have an intensity of infection of hundreds or thousands of these monogeneans (Musselius, 1967).

*Dactylogyrus iwanowi* Bykhovskii, 1957 *nomen nudum*

The host fish *Leuciscus brandti* is found along the entire coastline of the Sea of Japan, and the coasts of Sakhalin. This species and related species of fish in the Sea of Japan are the only marine representatives of the Cyprinidae (Berg, 1949). *Leuciscus brandti* has spawning migrations to the rivers, entering the Suifun and other rivers flowing into Peter the Great Bay, near Vladivostok, U.S.S.R., when the ice melts, and spawning in freshwater from May to September. The spent fish return to the sea during the summer, but immature fish occur in the river mouths the whole year around. The young winter in the river and descend to the sea at a length of 7 to 9 cm (Berg, 1949).

Bykhovskii (1957) described the life-cycle of *Dactylogyrus iwanowi* from fixed material. It is especially interesting in view of the more complex nature

of the life-cycle, owing to the migrations of the fish host, as compared with normal simple life-cycles found in the freshwater members of the genus *Dactylogyrus*. Bykhovskii found that infection by *D. iwanowi* took place during the river life of *L. brandti* and that mainly the young fish descending into the sea were infected. The worms lived on the fish for more than one year, reached maturity during the marine period of life, and during the approach of the fish to the rivers increased their rate of deposition of eggs. Bykhovskii noted that it remained to be explained whether oviposition occurred during the marine period of life of *D. iwanowi* and, if so, what was the fate of these eggs. Conditions in the sea excluded the possibility of larvae encountering the fish. Likewise, the secondary infection of adult *L. brandti* which occurred in freshwater was a rarer occurrence than the infection of the young, owing to lack of appropriate conditions for contact of the larvae with these older fish (Bykhovskii, 1957).

*Dactylogyrus lamellatus* Akhmerov, 1952

Molnár (1971a,b,c, 1972), working at the pond fish farms of Szarvas and Dinnyés, Hungary, investigated the biology of *D. lamellatus* in great detail. *D. lamellatus* was found to infect *Ctenopharyngodon idella* of all ages and sizes throughout the year (Molnár, 1971b). Infestation persisted during the winter, but was of a very low degree; fish carried only a few parasites and some were free of infection. During spring the incidence of infection tended to increase with the rise of temperature. *C. idella* five to seven years old kept until the end of the spawning season often became massively infected. With fry, hatched in early July, and studied from early August to December, the infections at first increased during August but declined by mid-October in a control group of fry and in a group exposed to infection by the presence of one-summer *C. idella* with infestations of *D. lamellatus*. In this latter group infection increased again towards December (Molnár, 1971b). The parasites occurred during the winter, but reinfection was impossible. Massive reinfection occurring during the spring was brought about not so much by eggs which overwintered, but by the reproduction of those dactylogyrids which had survived the winter (Molnár, 1971a). Musselius and Ptashuk (1970) stated that the eggs of *D. lamellatus* could overwinter at the bottom of dried ponds.

*Dactylogyrus macracanthus* Wegener, 1909

According to Bauer (1959a) the heaviest infection of *Tinca tinca* in western Europe was seen from April to June. Wilde (1935, 1937) observed that the time of embryonic development was determined by temperature. In March to April embryonic development took 10 to 13 days, in winter 18 to 20 days. The complete developmental cycle, egg to sexual maturity, took 16 days at 20°C–23°C and around 30 days at 13°C–14°C. Slow reproduction occurred during winter.

Komarova (1957) studied *D. macracanthus* on *T. tinca* in the Donets River, U.S.S.R. She found the worms all year, with maximum incidence in the spring (1952, 73·3%; 1953, 80·0%) and minimum in the winter of 1952 (33·3%) and during the summer of 1953 (20%).

*Dactylogyrus merus* Zaika, 1961

Studied by Molnár (1968) at Lake Balaton, Hungary, on *Phoxinus phoxinus*,

but the observations were not separated from those of *D. borealis* and *D. phoxini*. Found during April, June, July and October but not during the other months.

*Dactylogyrus minor* Wagener, 1857

Izyumova (1960) observed this species on *Rutilus rutilus* in the Rybinsk Reservoir, U.S.S.R. only during the autumn.

*Dactylogyrus nanus* Dogiel & Bykhovskii, 1934

Occurred on *Rutilus rutilus* at the Iriklin Reservoir, River Ural, U.S.S.R., from April to July, with maximum incidence 33% in May and June (Kashkovski, 1967). Not found during the other months. Izyumova (1964) noted that *D. nanus* occurred on the fishes predominantly in spring and summer. Rumyantsev (1972) showed that the lower the spring and early summer water temperatures in Lake Srednego Ku'to, Karelia, U.S.S.R., the greater the displacement of the infection peak.

*Dactylogyrus nasalis* Strelkov & Ha Ky, 1964

This species occurred in the nasal cavities of *Blicca bjoerkna*, *Rutilus rutilus* and *Scardinius erythrophthalmus* in lakes in the Pskov region and Karelia, U.S.S.R. (Strelkov and Ha Ky, 1964). Other monogeneans which occurred in this organ of fishes were listed by Smirnova *et al.* (1964). According to Strelkov *et al.* (1969), *D. nasalis* was most abundant on *Rutilus rutilus* in October and November at Vrevo Lake, near Leningrad, U.S.S.R. Yunchis (1974) showed that young *R. rutilus* at this lake were commonly infected but only at temperatures between 2°C and 14°C. The early stages of infection were found at first on the body surfaces and fins of the fish and later on the gills and in the nasal cavities.

*Dactylogyrus nobilis* Long & Yu, 1958

Bauer *et al.* (1969) stated that in the European part of the U.S.S.R., *D. nobilis* appeared on *Aristichthys nobilis* in late May or early June, its numbers increasing in June and July and decreasing sharply in October. Musselius and Ptashuk (1969) stated that *D. nobilis* required for its development a water temperature of 16°C–35°C, optimum 30°C. At the optimum temperature the numbers of viable eggs and hatched larvae were greatest. At 6°C–8°C the eggs were unable to develop (Musselius and Ptashuk, 1969).

*Dactylogyrus phoxini* Malevitskaya, 1949

Recorded on *Phoxinus phoxinus* in Lake Balaton, Hungary, in April, June, July and October, but not in the other months (Molnár, 1968). Not separated from *D. borealis* and *D. merus* in Table 1, of Molnár's paper.

*Dactylogyrus prostae* Molnár, 1964

Davies (1967) found *D. prostae* on *Leuciscus cephalus* in the River Lugg, Herefordshire, England, during February to August. In male fish the worm occurrence peaked in April and August, in female fish during March and July–August. During September to January the fish were uninfected.

*Dactylogyrus ramulosus* Malevitskaya, 1941

Found by Kashkovski (1967) on *Rutilus rutilus* in the Iriklin Reservoir,

River Ural, U.S.S.R., during February to May, but not in the other months. Maximum occurrence (26%) was in February and May.

*Dactylogyrus similis* (Wegener, 1907)

Izyumova (1960) found this species on *Rutilus rutilus* at the Rybinsk Reservoir, U.S.S.R. in winter (17·5%) and spring (38·0%). Komarova (1964) observed *D. similis* on *Blicca bjoerkna* in the Dnepr Delta, U.S.S.R., during March, and not in February, April, May, June and October. Rizvi (1964, 1969), at Rostherne Mere, Cheshire, England, examined *R. rutilus* during all months, and found *D. similis* from March to August. Maximum incidence was in April (40%) and May (36%), the infection declining thereafter. Mishra (1966) also examined *R. rutilus*, at the Shropshire Union Canal, Cheshire, England, and observed *D. similis* in March, May and June. No fish were infected during the other months. The maximum incidence was 6·2% in April. Kashkovski (1967), at the Iriklin Reservoir, Ural River, U.S.S.R., found *D. similis* on *R. rutilus* during April (6·6%) and May (40%). Infections were not seen during the other months.

The displacement of the infection peak of *D. similis* on *R. rutilus* in Lake Srednego Kuĭto, Karelia, U.S.S.R., by low spring and early summer temperatures was demonstrated by Rumyantsev (1972). In 1962, a cold spring and early summer displaced the infection to such an extent that it had not appeared by July, but in 1963, a warmer spring, *D. similis* infected 20% of the fish in May.

*Dactylogyrus simplicimalleata* Bykhovskii, 1931

Izyumova (1958b) found that at the Rybinsk Reservoir, U.S.S.R., the infection of *Pelecus cultratus* by *D. simplicimalleata* was more or less the same throughout the year, but on *Abramis brama* it was not found during the winter. She explained this difference in occurrence by the level of oxygen available in the different conditions in which the two species of fish overwinter.

Komarova (1964) examined *Pelecus cultratus* in the Dnepr Delta, U.S.S.R., in February, March, April–May, July–August and October. During all these months the incidence of *D. simplicimalleata* was between 80%–93·3%.

*Dactylogyrus sphyrna* Linstow, 1878

Izyumova (1960) found *D. sphyrna* all the year round at the Rybinsk Reservoir, U.S.S.R. Komarova (1964) also investigated the incidence of *D. sphyrna* in the Dnepr Delta, U.S.S.R.

*Blicca bjoerkna* were infected in all months examined, February to June, and October. Peak incidence was from April to June, with maximum intensity in May. *Rutilus rutilus heckeli* were infected from February to August, and in October. The other months were not investigated. Maximum incidence was in April and May, and the intensity more or less similar throughout. *Vimba vimba vimba* natio *carinata* were infected in February to July, and October. The remaining months were not investigated. Peak incidence was in April–May, with maximum intensity in June–July. Contrary to Komarova finding *D. sphyrna* during all months investigated, Kashkovski (1967) at the Iriklin Reservoir, River Ural, U.S.S.R., found this species on *R. rutilus* only in June and August. In England Mishra (1966) examined *R. rutilus* at the Shropshire

Union Canal, Cheshire, during all months. *D. sphyrna* was found from March to December, with maximum incidence in August. The overall infections were low in the fish studied by both Kashkovski and Mishra.

In Poland, Wierzbicka (1974), at Lake Dabie, near Szczecin, found *D. sphyrna* to be one of the commonest parasites of *Blicca bjoerkna*. The general incidence of infection was 64·5%. The incidence varied considerably according to the season of the year, reaching a peak of up to 100% in the spring and summer months. A considerable drop in incidence was found in the autumn and winter. The changes in intensity of infection were parallel to those of the incidence, which was highest in spring and summer, lowest in autumn and winter.

*Dactylogyrus vastator* Nybelin, 1924

*D. vastator* infects *Cyprinus carpio*, from the fry stage to adult fish. As Bykhovskii (1957) clearly stated, an important factor in the understanding of the life-cycle of *D. vastator* was that in natural conditions the levels of infection fluctuated not only with the season of the year, but also with the age of the fish. Lyaiman (1951b) showed that infection of fry first occurred after the tenth day of life and mainly during the first months of life. Izyumova (1953, 1956a, b) experimentally verified this situation. Nordquist (1925) and Nybelin (1925) observed maximum infection of *C. carpio* fry of 2–5 cm in length. The fry attained this length in the temperate zone of the U.S.S.R. from the end of June to the first half of July. Following this stage of maximum infection, there was a dramatic reduction in parasite abundance. Only single *D. vastator* were found on fingerling fish by autumn. Underdeveloped fingerlings, weighing 3–5 g, were most heavily infected (Bauer, 1959a). Towards the autumn and winter period *D. vastator* was found on the older *C. carpio*, but with a smaller incidence and intensity of infection.

Nordquist (1925), Nybelin (1925) and Wunder (1929) considered that *D. vastator* was not found on the fish in winter, but that a new infection arose from overwintered eggs. Lyaiman (1948, 1951a) showed that *C. carpio* was infected the whole year around in the U.S.S.R. However, the eggs can overwinter, as they hatched in about 100 days at 4°C (Bauer, 1959a).

The sudden decrease in incidence and intensity of *D. vastator* in the second half of the summer was most likely owing to the formation of epithelial outgrowths on the gill lamellae, on which the parasites were unable to survive and so died (Wunder, 1929; Paperna, 1964b; Uspenskaya, 1961). Similar changes in the gills have also been described for other *Dactylogyrus* species (*D. extensus*, Bauer and Nikol'skaya, 1954; Paperna, 1964b; Prost 1963; *D. lamellatus*, Molnár, 1972; *D. macracanthus*, Wilde, 1937). What had not been clarified was why reinfection did not recur during the second half of the summer. Bauer (1959a) explained this by the behaviour of the larvae of *D. vastator*, living in the warmer superficial layers of water, whereas the fish as they reached this period of life sank to the bottom to feed on benthos, and thus the contact between the larvae and the fish was prevented.

In the central zone of the U.S.S.R. the optimum temperatures for the life-cycle of *D. vastator* were 22°C–24°C (Bauer *et al.*, 1969). In Israel Paperna

(1963) found a rather different range of optimum temperatures, 24°C–28°C.

Paperna (1963) found *D. vastator* on *C. carpio* fry from April to June only. At the end of June the fry were in excess of 60 mm in length and were resistant to infection by *D. vastator*. Accordingly, to enable the infections to be carried over from one year to the next, Paperna postulated the existence of diapausing eggs. Such variability in developmental potential of eggs was reported by Nordquist (1925), Nybelin (1925) and Wunder (1929). A drop in temperature induced the larger "winter eggs". Kulwieć (1929), Spiczakow (1930) and Bykhovskii (1933a) questioned the existence of larger eggs or of diapausing eggs, but nonetheless, Paperna was able to show that at least some eggs did not develop immediately to produce larvae.

*Dactylogyrus vistulae* Prost, 1957

Davies (1967) found *D. vistulae* on *Leuciscus cephalus* in the River Lugg, Herefordshire, England, from February to August, but not from September to January. Maximum incidence was in April (87%). Worms had eggs in the uterus during all months, but a higher percentage carried eggs from February to May (44%–64%) than from June to August (20%–21%).

*Dactylogyrus wunderi* Bykhovskii, 1931

Reported in the Rybinsk Reservoir, U.S.S.R., as occurring on the fishes predominantly in spring and summer, and usually disappearing in autumn and winter (Izyumova, 1964). Komarova (1964) in the Dnepr Delta, U.S.S.R., examined *Abramis brama* in February, but found no *D. wunderi*. In March 6·6% were infected, and the incidence climbed through April and May to a maximum in June of 80%. Worms were present in July–August (28·5%) and October (40·0%). The remaining months were not investigated. Intensity of infection showed no marked seasonal change.

In England, at the Shropshire Union Canal, Cheshire, Mishra (1966) examined *A. brama* in January and April to August. In January no worms were found. In April and May 50% incidences, in June and July 100% incidences, and in August 66·6% incidence were observed.

Wierzbicka (1974) found *D. wunderi* during all months investigated at Lake Dabie, near Szczecin, Poland. The incidence on *A. brama* reached 87·1% in March and remained high (80%–100%) from March to October. Only in November and December did the incidence decrease to 50%. Fish were not examined in January and February. Intensity of infection was lowest in December.

*Dactylogyrus zandti* Bykhovskii, 1933

Observed by Komarova (1964) on *Abramis brama* in the Dnepr Delta, U.S.S.R., but only during May, at a low incidence of 6·6%. It was not found in February to April, June to August, or October. Wierzbicka (1974) at Lake Dabie, near Szczecin, Poland, found an overall incidence of 64·5%. Peak incidence of 100% occurred in summer, decreasing in the autumn, winter and spring. Deviations in seasonal fluctuation were observed from year to year.

*Lyrodiscus seminolensis* Rogers, 1967

Studied by Rawson and Rogers (1972b) on *Lepomis macrochirus* at the Walter

F. George Reservoir, Alabama, U.S.A. It belonged to their Group 2, large species most abundant during the spring. *L. seminolensis* was observed in the winter months but it did not reach maximum occurrence until May. The population declined by September to an undetectable level, and remained so for the duration of the investigation.

*Pseudacolpenteron pavlovskii* Bykhovskii & Gussev, 1955

Margaritov (1965) found *P. pavlovskii* on *Cyprinus carpio* in fish farms in Bulgaria throughout the year.

*Urocleidus acer* (Mueller, 1936)

Rawson and Rogers (1972b) placed this species in their Group 3, small species abundant during the summer but with a prewinter peak at water temperatures near 10°C. In the Walter F. George Reservoir, Alabama, U.S.A., *Lepomis macrochirus* was infected at a low population level during the winter months prior to the spring increase in temperatures above 26°C. High populations of *U. acer* occurred in June and September at temperatures of 27°C–29°C and the population was at a moderate level during the warmer summer months when temperatures were above 30°C. The population declined to a low level during the period of maximum temperature decline in the autumn, but increased again to a high level at temperatures near 10°C, decreasing at lower temperatures.

*Urocleidus adspectus* Mueller, 1936

Tedla and Fernando (1969) examined the occurrence of *U. adspectus* on *Perca flavescens* from the Bay of Quinte, Lake Ontario, Canada. The incidence did not show any significant seasonal change. The intensity of infection reached a pronounced peak in August and September and returned to a relatively constant level from December onward. Tedla and Fernando stated that although the life-cycle of *U. adspectus* was not known, their findings indicated that its breeding might be seasonal. When the water temperature increased in early summer some adult worms, which survived the winter on the host, died after depositing eggs. This may have accounted for the slight drop in numbers from May to July. The post-embryonic period for *U. adspectus* might be very short and this new generation could account for the high intensity of infection in August and September. When the water cooled in autumn, egg laying could be impaired or continue at a low level. These eggs might pass the winter at the bottom of the lake and hatch during the late spring increase in water temperature. This generation might also contribute to the high intensity during the summer. During the winter, established worms could be gradually lost, owing to adverse conditions (Tedla and Fernando, 1969).

*Urocleidus dispar* (Mueller, 1936)

Also investigated on *Lepomis macrochirus* at the Walter F. George Reservoir, Alabama, U.S.A., by Rawson and Rogers (1972b), and belonging to their Group 3, small species abundant during the summer but with a prewinter peak at a water temperature around 10°C. The population was low during the winter months prior to the spring increase in water temperatures above 26°C. During the summer warm months the population oscillated regularly, reaching high levels at two-month intervals from June to October when the temperature declined to 26°C. During the autumn period of maximum temperature drop

the population declined to a low level, but increased again to a high level when the temperature reached 10°C, declining yet again thereafter at a January low temperature of 7°C–8°C.

*Urocleidus ferox* Mueller, 1934

Investigated on *Lepomis macrochirus* in Sacramento County, California, U.S.A., by Crane and Mizelle (1968). An annual average worm population was 15·1 individuals per fish. Its intensity fluctuated markedly, showing peaks in August, January and April with averages per fish of 18·2, 22·2 and 26 parasites, respectively. The August and April peaks were positively related to temperature, but that for January occurred when the temperature was at the annual low (8°C). Observation of developmental forms of *U. ferox* suggested that the continuous presence of *U. ferox* on the fish was not entirely attributable to adults surviving for extended periods, as the life-cycle proceeded continuously in California.

In the Walter F. George Reservoir, Alabama, U.S.A., Rawson and Rogers (1972b) also examined *U. ferox* on *L. macrochirus*. They included the parasite in their Group 3, small species abundant during the summer but with a pre-winter peak at water temperatures around 10°C. In December and January the population was low, and it did not increase significantly until the surface water temperature rose above 25°C in June. In July it reached a peak, and declined to a moderate level. In the autumn, when the temperature dropped below 26°C, and entered its period of maximum decline, the worm population decreased to the low levels of the previous winter and spring. The population increased rapidly to a high level as the surface temperature approached 10°C, and at the termination of the investigation was at a moderate level when temperatures had gone below 10°C.

*Urocleidus furcatus* (Mueller, 1937)

Rawson and Rogers (1972a) examined *U. furcatus* on *Micropterus salmoides* in the Walter F. George Reservoir, Alabama, U.S.A. Intensity of infection was at the lowest level (14) in January and increased to a moderate level (79–156) and remained at this level until June. After the spring period of maximum temperature increase, intensity increased in June then declined to the former levels. In mid-September intensity increased to a peak (301). Summer peaks in intensity were associated with a surface water temperature near 28°C. The intensity declined in October and November during the period of maximum temperature decrease, but reached its maximum level (320) in December at a water temperature of 9°C. In January intensity declined again.

*Urocleidus principalis* (Mizelle, 1936)

Investigated on *Micropterus salmoides* at the Walter F. George Reservoir, Alabama, U.S.A., by Rawson and Rogers (1972a). Intensity of infection by *U. principalis* varied during the winter months (14–150) but increased to a peak of 302 in mid-April after the period of maximum change of the surface water temperature. Intensity declined until late May prior to increasing to a peak (394) in June when surface water temperature was 28°C. It then declined to a moderate level (167–276) and remained at this level during the summer. In September intensity reached 459 at a temperature of 28°C, and during the

autumn period of maximum temperature fall intensity declined steadily. In December, at 9°C, intensity again increased (299) near to the level of the previous spring peak. In January intensity decreased to the low level (58) of the previous winter.

(b) *Family Diplectanidae*

No seasonal studies of the freshwater species known to the author.

(c) *Family Protogyrodactylidae*

No seasonal studies of the members of this family known to the author.

(d) *Family Calceostomatidae*

No seasonal studies of the freshwater species known to the author.

(e) *Family Capsalidae*

No seasonal studies of the genus *Nitzschia*, which occurs on migratory Acipenseriformes, are known to the author.

2. *Order Gyrodactylidea*

(a) *Family Gyrodactylidae*

The gyrodactylids are viviparous. Bykhovskii (1957) gave a full account of the details of the life-cycle and the accretion in number of progeny of one individual of *Gyrodactylus rarus* during 20 days. Braun (1966) studied *G. wageneri* and found that daughter worms continued to reproduce without interruption for at least 20 generations, and more recently Lester and Adams (1974a,b) studied experimental populations of *Gyrodactylus alexanderi* on *Gasterosteus aculeatus*.

*Gyrodactylus alexanderi* Mizelle & Kritsky, 1967

Found by Lester (1974) on *Gasterosteus aculeatus* in a number of habitats near Vancouver, British Columbia, Canada, during all months of the year. No details were provided.

*Gyrodactylus anguillae* Ergens, 1960

Malmberg (1970) found *G. anguillae* on elvers of *Anguilla anguilla* in the River Morup, Falkenberg, Halland, Sweden. The elvers were about 7 cm long and were part of the annual run of elvers into the River Morup. Malmberg examined elvers in the summer of 1959 and during May, June and July, 1960. Infected elvers were seen in June and July. Malmberg demonstrated that *G. anguillae* can live in salt, brackish or freshwater conditions. The worms were found only on elvers.

As the run of elvers was an annual event, Malmberg speculated concerning the origin of the infection of *G. anguillae*. The question was, were the elvers infected in the sea or when they migrated into freshwater? If *G. anguillae* was species-specific to *A. anguilla* and if the elvers were uninfected until they reached freshwater, then they must have become infected through coming into contact with larger infected river specimens of *A. anguilla*. Another possibility was that they were infected in coastal waters by coming into contact with infected eels. Malmberg had found no specimens of *G. anguillae* on *A. anguilla* larger than elvers in fresh, brackish or salt water. According to Malmberg

this may indicate that the infection of the elvers actually occurred before the elvers reached the Swedish coast and also that *G. anguillae* was a species living in waters of warmer latitudes than those of Sweden. If correct, all the *G. anguillae* probably died after the elvers had been in Swedish waters for some time. Malmberg considered it was possible that the fry of *A. anguilla* were infected at the spawning place, but this would have to be through contact with infected adult *A. anguilla*, if *G. anguillae* was species-specific (Malmberg, 1970).

The above speculation concerning the time of infection of the elvers by *G. anguillae* was directly relevant to the seasonal occurrence of the species. As no resting phase has been reported for gyrodactylids, these parasites must be assumed, in the absence of contrary evidence, to transfer direct from one host to the next. With stages of the host life-cycle having a marked seasonal pattern, as with elver migrations, one must seek an alternative host for the parasites for the remainder of the year. Ergens (1960), who described *G. anguillae*, did not indicate the size of *A. anguilla* from which he obtained his material, hence no additional information was available from that record.

*Gyrodactylus aphyae* Malmberg, 1956

One of five species which occurred on *Phoxinus phoxinus* at Lake Balaton, Hungary (Molnár, 1968). Unfortunately the species were not separated in the table showing incidence and intensity of infection. Molnár indicated that the infection was at a maximum in the spring and at a minimum in the autumn, but worms were present in all months.

*Gyrodactylus arcuatus* Bykhovskii, 1933

Studied by Banina and Isakov (1972) at a Neva Delta reservoir, near Leningrad, U.S.S.R., on *Gasterosteus aculeatus* and *Pungitius pungitius*. The fish were examined during all months except December and January when ice covered the water. No infections were found on either species of fish in February and March, but in April 6·7% of *G. aculeatus* were infected. No infections were found in May but both fishes were infected in June. At the beginning of June the incidence was *G. aculeatus* 26·7%, *P. pungitius*, 6·25%; at the middle of June *G. aculeatus* 32·0%; and at the end of June *P. pungitius* 31·0%. In July the incidence levels were *G. aculeatus* 37·0% and *P. pungitius* 25·0%, with intensitities of 10·2 and 1·7 respectively. No *G. aculeatus* were examined in August, but the September incidence was 30%. In October incidence rose to 40%, to fall again to 10% in November. In August 2·0% of *P. pungitius* were infected, 23·1% in September, 11·1% in October and none in November. Maximum occurrence of *G. arcuatus* on both fish species was at a period of high temperature (17°C–18°C).

*Gyrodactylus arcuatus* Bykhovskii *sensu* Bykhovskii & Polyanski, 1953

Regarded as a separate species from *G. arcuatus* Bykhovskii, 1933 by Malmberg (1970). Malmberg studied the intensity of infection on *Gasterosteus aculeatus* at Nämdo, Baltic Sea, Sweden throughout the year, except February and March, when the water was frozen. Several parasites were found on two fish on 1st April. During May to August the parasite was common, but from

September to the time when the ice formed it was rarely found. No worms were found on a specimen of *G. aculeatus* collected on 2nd January. The rare occurrence of *G. arcuatus* from the autumn to the beginning of spring seemed also to apply to the Swedish West Coast, judged from living *G. aculeatus* which were sent from Kristineberg several times during this season. Temperatures of 2°C–30°C were suitable for *G. arcuatus* to be kept experimentally. One *G. aculeatus* was maintained at about 4°C–5°C from December to May, and five specimens of *G. arcuatus* were found on it (Malmberg, 1970).

*Gyrodactylus bychowskyi* Sproston, 1946

Regarded as a synonym of *G. arcuatus* Bykhovskii *sensu* Bykhovskii and Polyanski, 1953 by Malmberg (1970). Also studied by Banina and Isakov (1972) on *Gasterosteus aculeatus* and *Pungitius pungitius* in a Neva Delta reservoir, U.S.S.R. All months except December and January were sampled; *G. bychowskyi* was found in mid-June to September only. *G. aculeatus* incidence: mid-June 9%, July 53% and September 10%, maximum intensity July, 14·6. *P. pungitius* incidence: July 25·0%, August 1% and September 15%, maximum intensity July, 3·7. As *G. bychowskyi* occurred in four months only, one must speculate how the species was able to survive through the remainder of the year.

*Gyrodactylus elegans* Nordmann, 1832

Observed at Lake Dabie, near Szczecin, Poland, by Wierzbicka (1974), on *Abramis ballerus*, *Abramis brama* and *Blicca bjoerkna*. It occurred most frequently in the summer on *A. ballerus*. The maximum incidence was in June and July, 1970 (28·6% and 23·8% respectively). From October to April it was not found at all. On *A. brama* it was seen mostly in the spring and summer months. Peak incidence was in June and July, 1970 (66·7% and 63·6% respectively). Again it was not observed in the winter months and early spring. On *B. bjoerkna* it was found in the spring and summer months. Maximum incidence was in June and July, 1970 (58·3% and 50% respectively). In the autumn and winter months no *G. elegans* were found on *B. bjoerkna*. On all three species of fish, intensity of infection varied from single to very numerous specimens. Ivasik (1957) observed low infections on *Cyprinus carpio* during the winter in pond fisheries in the Ukraine.

*Gyrodactylus laevis* Malmberg, 1956

Infected *Abramis ballerus* and *Blicca bjoerkna* at Lake Dabie, near Szczecin, Poland (Wierzbicka, 1974). It was found only during the summer months on both fishes, with an overall incidence of 4·1% on *A. ballerus* and 3·5% on *B. bjoerkna*.

Molnár (1968) found *G. laevis* on *Phoxinus phoxinus* from Lake Balaton, Hungary. Gyrodactylids occurred on the fish during all months of the year, but in his table giving incidence and intensity of infection the five species found were not separated.

*Gyrodactylus lucii* Kulakovskaya, 1952

Rautskis (1970b) examined this species on *Esox lucius* in Lake Dusia,

Lithuanian S.S.R. Its occurrence was January to March, 0; April–May, 62·5%; June–July, 53·3%; October–November 26·6%.

*Gyrodactylus macrochiri* Hoffman & Putz, 1964

Studied by Rawson and Rogers (1973) on *Lepomis macrochirus* and *Micropterus salmoides* at the Walter F. George Reservoir, Alabama, U.S.A. The worm population intensity on *L. macrochirus* was at a low level at the start of the study in December, and was relatively stable during the winter and spring. It rose to a peak intensity (361) in early June at a temperature of about 28°C. The population of worms decreased to low levels during the warmest water temperatures of the year, above 30°C, from July to September, but peaked again in September at 27°C. Both the peaks of intensity of occurrence of *G. macrochiri* were at water temperatures near 24°C–27°C. The intensity fell rapidly in October, but increased again during November and December to reach a high level in January at the end of the study.

On *M. salmoides*, the population intensity was also at a low level at the commencement of the study in December, but increased rapidly in early February to an extremely high level (1398). As the water temperature fell below 10°C the intensity declined to a low level in early March, but with the rapid spring rise in temperature an increase in the population intensity occurred (April–May) at temperatures of 20°C–25°C. At temperatures above 25°C the population of *G. macrochiri* fell to a low level, and remained at this intensity throughout the summer and autumn (June to November), but in December it increased as the water temperature fell below 12°C.

Rawson and Rogers remarked that *G. macrochiri* demonstrated an ability to reproduce in a short period of time to high population intensity levels on both species of fish. The high population levels of *G. macrochiri* occurred in three circumstances: during the spring reproductive period of the fish, during the autumn when temperatures were similar to those during the spring reproductive period, and during the winter when temperatures were below 10°C.

*Gyrodactylus macronychus* Malmberg, 1956

Molnár (1968) found *G. macronychus* on *Phoxinus phoxinus* from Lake Balaton, Hungary. Gyrodactylids were present on the fish during all months of the year, but his table indicating incidence and intensity of infection did not separate the five species which occurred.

*Gyrodactylus parvicopula* Bykhovskii, 1933

Kashkovski (1967) examined *Rutilus rutilus* at the Iriklin Reservoir, River Ural, U.S.S.R., during all months of the year. *G. parvicopula* occurred in May (6·6%). *R. rutilus* was not indicated as a host for *G. parvicopula* by Bykhovskaya-Pavlovskaya *et al.* (1962), hence it may be suggested that *R. rutilus* was an accidental host, perhaps infected at a time of maximum occurrence of *G. parvicopula* on its usual hosts *Abramis brama*, *Blicca bjoerkna* and *Vimba vimba*.

*Gyrodactylus phoxini* Malmberg, 1956

As with *G. macronychus*, Molnár (1968) found *G. phoxini* on *Phoxinus*

*phoxinus* at Lake Balaton, Hungary. The same comment applies as stated above, concerning incidence and intensity of infection.

*Gyrodactylus pungitii* Malmberg, 1964

Found by Malmberg (1970) in Sweden on *Pungitius pungitius* during all months. He noted seasonal variations in morphology of *G. pungitii* in both the fresh and brackish water forms. The differences were greatest during the colder water period; the summer forms were much alike, even difficult to separate. Kept at 5°C–8°C for several months, it was possible both to retard the change from winter to summer form and to bring about the change from summer to winter form.

*Gyrodactylus rarus* Wegener, 1910

Bi-monthly samples of *Gasterosteus aculeatus* were examined from a pond at Baildon Moor, Yorkshire, England (Chappell, 1969). *G. rarus* was found in each sample; the incidence was high in samples collected in September (93%), November (79%), January (99%), March (100%) and May–June (82%), but very low in August (0·8%). Intensity, mean (and maximum), varied from sample to sample: September 6·7 (25), November 6·1 (31), January 11·5 (61), March 23·4 (84), May–June 6·2 (56) and August 2·0 (2). Chappell made measurements of whole-mount preparations of the worms which indicated that the size and degree of maturity of the parasites varied with season. The percentage of *G. rarus* bearing developing embryos showed a distinct seasonal pattern. No worm was embryonated in September, but from November to June an increasing percentage of worms was embryonated: November 50%, January 76%, March 75% and May–June 86%. The August worms, two only, were not embryonated. Chappell suggested that if the annual cycle noted above was repeated from year to year, with high infection levels from September to June, followed by severe depletion of the population in August, then it might be that the infection of newly hatched *G. aculeatus* occurred after the parasite was transferred from the breeding fish to the deposited eggs.

Malmberg (1970), in Sweden, kept *G. rarus* throughout the year at laboratory temperatures of 4°C–10°C and found it naturally on *Pungitius pungitius* most of the year.

Banina and Isakov (1972) found *G. rarus* on *Gasterosteus aculeatus* and *Pungitius pungitius* in a Neva Delta reservoir, near Leningrad, U.S.S.R. These authors suggested that *G. rarus* was a warmth-loving species. They examined fish from February to November, but not during December and January, when ice covered the water. On *G. aculeatus* they found *G. rarus* in July (6%), and on *P. pungitius* in July (12%) and September (30%).

*Gyrodactylus* species

Bauer *et al.* (1969) list *G. elegans*, *G. medius*, *G. cyprini* and *G. sprostonae* from *Cyprinus carpio* and *G. ctenopharyngodonis* from *Ctenopharyngodon idella*. They noted that epizootics were reported mainly from the southern parts of the U.S.S.R. In the central part of the country large numbers of these parasites may be found in wintering ponds towards the spring. The fact that *Gyrodactylus* occurred mainly in the southern areas indicated that it preferred higher temper-

atures. *G. elegans* was apparently more warmth-loving than *G. medius*. *G. elegans* was commoner than *G. medius* in years with a greater number of hot days.

3. *Order Tetraonchidea*

(a) *Family Tetraonchidae*

*Tetraonchus borealis* (Olsson, 1893)

This species was investigated on *Thymallus thymallus* from the River Lugg, Herefordshire, England (Davies, 1967). Samples of these fish were examined in all months except January and September. No worms were found in February and November; in the remaining months the incidences were March 33%, April 88%, May 63%, June 47%, July 80%, August 20%, October 4% and December 7%. The percentages of worms with eggs *in utero* were noted for each month: March 13%, April 3%, May 25%, June 10%, July 13%; in August, October and December no eggs were seen. Peaks of intensity of infection occurred in April and July.

*Tetraonchus monenteron* Diesing, 1858

Markova (1958) studied *T. monenteron* on *Esox lucius* in the Oka River, U.S.S.R. The parasite had maximum incidence in August, was present March to December, but was not found in January and February. At Llyn Tegid, Wales, Chubb (1961) found *T. monenteron* on *E. lucius* of length greater than 40 cm. Fish of this length upwards were infected throughout the year. Rizvi (1964, 1969) at Rostherne Mere, Cheshire, Mishra (1966) at the Shropshire Union Canal, Cheshire, and Davies (1967) at the River Lugg, Herefordshire, England, also found *E. lucius* to be infected during the whole year. Rizvi, in particular, found a high incidence throughout: 100% in February, April, August and October, 93% in December, 89% in March and May, 83% in June, 78% in November, 75% in July, 50% in September and 40% in January.

Izyumova (1964) found *E. lucius* at the Rybinsk Reservoir, U.S.S.R., infected all the year round. Komarova (1964) examined *E. lucius* in the Dnepr Delta, U.S.S.R., in February, March, April–May and October. *T. monenteron* was present in each month. Rautskis (1970b), at Lake Dusia, Lithuanian S.S.R., also found *T. monenteron* on *E. lucius* throughout the year.

## B. SUBCLASS OLIGONCHOINEA

1. *Order Diclybothriidea*

(a) *Family Diclybothriidae*

*Diclybothrium armatum* Leuckart, 1835

Bauer (1959b) collected material on the infection of *Acipenser ruthenus* by *D. armatum* from April to October. An inverse relationship between intensity and the water temperature was observed. A rise in temperature during the summer was associated with a fall in infection of *A. ruthenus*. The autumn fall in temperature was associated with an increase in infection.

Sexually immature *D. armatum* were found throughout the period of investigation. Although infection of *A. ruthenus* occurred throughout, Bauer

postulated that the winter was the main infection period, as evidenced by the large number of young *D. armatum*, from recently attached to mature worms with formed vitelline glands, which were found in the spring. The explanation offered by Bauer for the main infection occurring at a time when ecological conditions were unfavourable, as hatching was delayed and larval development slow near 0°C, was related to a pattern of behaviour of *A. ruthenus*. This species of fish gathered together, for hibernation, as the autumn water temperatures fell. Many were collected in one place, close to each other, in an inactive state. Bauer noted that this assembly of *A. ruthenus* allowed the transfer of infection from one fish to another. The acipenserids did not assemble during other seasons, nor was an increase in infection found at the spawning period.

In the life-cycle of *D. armatum*, main infection occurred during late autumn or winter, on hibernating fish. Parasite growth was slow during winter, but accelerated as the spring water temperatures increased. Most *D. armatum* became sexually mature in June and died during the summer months. A few worms reached sexual maturity during their first summer, to provide infections in summer and early autumn, and hibernated overwinter to die at the start of the following summer. The life of these *D. armatum* was one year, but the one-year cycle was not so sharply apparent as in many freshwater fish parasites with indirect life-cycles (Bauer, 1959b).

Temperature was shown to be the most important factor influencing *D. armatum*. Growth, reproduction and postembryonic development were increased at 18°C–20°C, but a higher temperature inhibited all stages of development. Lower temperatures slowed down all developmental processes, but the duration of life of the infective larva was increased, thereby facilitating infection transfer from fish to fish during the winter period of hibernation. The transition from dark to light, at dawn, was shown to stimulate hatching (Bauer, 1959b).

2. *Order Mazocraeidea*

(a) *Family Mazocraeidae*

*Mazocraes alosae* Hermann, 1782 occurred on Clupeidae which migrated into freshwaters of the U.S.S.R. (Bykhovskaya-Pavlovskaya *et al.*, 1962). Bykhovskii (1933b, 1957) studied the life-cycle in the Caspian Sea.

(b) *Family Discocotylidae*

*Diplozoon bliccae* (*D. paradoxum bliccae* Reichenbach-Klinke, 1961)

Found by Komarova (1964) on *Blicca bjoerkna* in the Dnepr Delta, U.S.S.R., in all months fish were examined (February to June, October). Maximum incidence (31·2%) and intensity of infection (7) were found in June.

*Diplozoon gussevi* Gläser & Gläser, 1964

Among one of the most frequent parasites of *Blicca bjoerkna* in Lake Dabie, near Szczecin, Poland (Wierzbicka, 1974), The overall incidence was 62%, mean intensity 3·22, range 1–14 parasites. It showed, despite sample deviations, a clear seasonal occurrence. Maximum incidence, to 100%, was found in July and August. Lowest incidence, 10%, was seen in autumn and early winter. Changes in intensity of infection followed parallel to those of incidence.

*Diplozoon homoion* Bykhovskii & Nagibina, 1959

Recorded on *Rutilus rutilus* at the Iriklin Reservoir, River Ural, U.S.S.R., from January to May (6·6% incidence, except February 13%), but not at all from June to December (Kashkovski, 1967).

*Diplozoon markewitschi* Bykhovskii, Gintovt, & Koval, 1964

On *Vimba vimba vimba* natio *carinata* in the Dnepr Delta, U.S.S.R. (Komarova, 1964), where fish were examined February to July, and in October. Incidence increased from February (20%) to June–July (66·6%). In October it was 13·2%. Intensity was highest March to July, when the incidence was also high.

*Diplozoon nagibinae* Gläser, 1965

Wierzbicka (1974) studied *D. nagibinae* on *Abramis ballerus* from Lake Dabie, near Szczecin, Poland. Overall, 81·3% of the fish were infected, and the intensity was also high, mean 5·34, range 1–43 worms. Peak incidence was found in summer and early autumn, up to 100%, and the lowest incidence (18·7% and 29·4% respectively) in November and December 1970.

*Diplozoon nipponicum* Goto, 1891

Parasitic on *Cyprinus carpio* and *Carassius carassius*, and studied by Kamegai (1970) in Japan. It occurred on the fish throughout the year. Kamegai described the detailed development of the reproductive organs during the year. He recognised four developmental stages of the testis and six of the ovary. In the autumn, after October, the cells of the testis appeared for the first time in the posterior half of the body. The cells were not composed of lobes, but were scattered in the posterior half of the body (T–I). Gradually lobes were formed (T–II), and at the end of winter, February–March, the testis was completely formed. There were three or more lobes and the uppermost one in contact with the ovary stained deeply (T–III). This condition continued until the end of the summer. In September a degeneration of the testis, seen as breaking of the lobes, occurred.

The ovarian cells appeared at the end of autumn, but only one or a few cells (O–I). The number of ova increased very gradually during the winter, to form a lobe at the end of the winter (O–II). During April and May the ovary increased in size suddenly (O–III). Many ova were seen; the ovary was in two parts, one cylindrical, the other oval. At the distal end of the ovary large ova were seen. At this stage the ovary was over one-quarter of the width of the posterior half of the worm. This condition continued until the end of August. Kamegai sometimes saw a degenerated ovary (O–IV), with spaces between the ova, in part of the organ. Stage O–V was a further degenerative phase, seen during June and September. No ovary (O–VI) was present in many (up to 75%) of worms during the winter months.

Oviposition started in April, continued during the summer and ended in September or October. Kamegai was unable to observe the fate of the worms after a season of oviposition.

*Diplozoon paradoxum* Nordmann, 1832

Until 1959, it was considered that a single species, *D. paradoxum*, was found

on all European cyprinid fishes. Bykhovskii and Nagibina (1959) concluded that *D. paradoxum* comprised several species, and accordingly erected three additional species: *D. homoion* from *Rutilus rutilus*, *D. megan* from *Leuciscus idus* and *D. pavlovskii* from *Aspius aspius*. *D. paradoxum* was considered to be a parasite of *Abramis brama*. Subsequently, further species have been described. The position has not yet been stabilised, as some species described have subsequently been reduced to subspecies, for instance *D. gracile* Reichenbach-Klinke, 1961, to *D. homoion gracile* by Oliver and Reichenbach-Klinke (1973). On some host fish, for example *Rutilus rutilus*, parasitised by *D. homoion* according to Bykhovskii and Nagibina (1959), a second species has been described, *D. rutili* Gläser, 1967. Karyological studies (Koroleva, 1968a,b, 1969) and comparative chaetotaxy of the larvae (Euzet and Lambert, 1974) promise to throw further light on the question of species validity in due time. In this account of seasonal information for *D. paradoxum* this author is not qualified to attempt to decide whether or not one or more species are involved. If the original authority named the material as *D. paradoxum*, they are included as *D. paradoxum*.

Zeller (1872) studied *D. paradoxum* on *Phoxinus phoxinus* in Germany. In winter adult worms occurred on the fish, but their genital systems were rudimentary. A rapid development of vitellaria and ova occurred in spring, or experimentally by the transfer of an infected fish into a warm aquarium during the winter, when egg formation had commenced by the 5th or 6th day.

Bykhovskii (1957) examined worms during the winter and found a fully developed sexual system, which functioned in the spring without any accelerated development of sex cells. Reproduction began in spring, and continued all summer, but the intensity of deposition of eggs was maximum in May and June. The eggs remained on the host gills, and Zeller counted up to 100 on a *Phoxinus phoxinus* with three parasites. The larvae emerged by the 9th–10th day (Bykhovskii) or the 12th–17th day (Zeller). The diporpa larvae settled on fish gills, living singly and growing slowly. After they developed two or three attachment clamps, growth ceased if they failed to meet a similar larva. If they met, they united in pairs, and developed as a pair subsequently. Single diporpa larvae died before winter. Paired individuals reached sexual maturity the following spring.

Izyumova (1964), from fish studied at the Rybinsk Reservoir, U.S.S.R., noted *Diplozoon* of fish. The lowest incidence on *Abramis brama* occurred in autumn and winter, on *Blicca bjoerkna* in summer and on *Rutilus rutilus* in spring. Izyumova considered this to mean that there were several species of *Diplozoon* present in her samples.

Komarova (1964) studied the incidence and intensity of occurrence of *D. paradoxum* on *Abramis brama* and *Rutilus rutilus heckeli* in the Dnepr Delta, U.S.S.R., from February to August and in October. *R. r. heckeli* was clearly not an important host as it was infected (6·6%) only in June. *A. brama* was infected in each month with minimum incidence in February (20%) and maximum in April (93·3%). The maximum intensity was also in April, range 1–17 worm pairs.

In Poland at Lake Dabie, near Szczecin, Wierzbicka (1974) found *A. brama*

infected throughout the year. In all, 70·8% were infected. The incidence of infection was high throughout, and only in the winter and early spring was there a fall in incidence.

Bovet (1967), at Lakes Neuchâtel, Bienne and Morat in Switzerland, found *D. paradoxum* on *Abramis brama*, *Blicca bjoerkna* and *Rutilus rutilus*. He designated his material as *D. paradoxum paradoxum* from *A. brama* and *D. paradoxum homoion* from *R. rutilus*. In a very detailed study, much information was provided concerning the biology of *D. paradoxum*. Of relevance here, at laboratory temperatures he found that the eggs hatched in a minimum of 5 days, average 15, maximum 17 days. The development of the embryo virtually ceased at 4°C. Eggs kept at this temperature remained viable, but on warming, 80%–100% hatched after 1½ months, 50% after 2 months, and only 2% after 2½ months. He concluded that the eggs produced during the cold months of the year contributed little to the maintenance of the species. Confirmation was found by the natural occurrence of the first diporpae on fish in July and August. The oncomiracidium life was shown to be 6 h at 18°C–20°C. In nature it was infective for about 4–6 h. They showed positive phototaxis, ascending 20 cm/min: about 10 m in the first hour. Diporpae were found on the fish in Lake Neuchâtel from the start of July to the end of November. Development speed was a function of temperature. A temperature of 24°C–26°C killed *D. paradoxum*, but not the fish. The longevity of the paired parasites was at least 2 years on *A. brama* and *R. rutilus*, as shown by three sizes: diporpae of the current season, a pair from the previous summer, and a larger pair from the summer 2 years earlier. The sexual cycle of development was annual.

Halvorsen (1969) studied *Diplozoon* on *Abramis brama*, *Rutilus rutilus* and a hybrid between the two species. He regarded *D. paradoxum* and *D. homoion* as being one species, *D. paradoxum*. There was a great uniformity in the length of the worm pairs at all times, even in August, after the main reproductive period. Size increased in May and June, reached a maximum in June, and decreased in July and August. Halvorsen concluded that most *D. paradoxum* lived about one year in Norway. In a subsequent publication (1972) Halvorsen gave details of the seasonal cycles of parasites of fish in the River Glomma. *D. paradoxum* was found on *R. rutilus* in all the months the fish were caught. On *R. rutilus* and *A. brama* the number of gravid *D. paradoxum* increased sharply from May to June, then decreased. Egg production was during the period April to November. The new generation of paired worms was established during the summer and the worms from the previous year died during this season. The spring rise in water temperature initiated sexual activity of the worms.

In England, at the Shropshire Union Canal, Cheshire, Mishra (1966) examined *D. paradoxum* on *Abramis brama* and *Rutilus rutilus*. Only a small number of *A. brama* were available, but the maximum incidence of *D. paradoxum* was during July and August. *R. rutilus* were infected throughout the year, with a minimum incidence in January (6%) and maximum in July (30%) Worm pairs with eggs were observed from March to July, not in August, but again in September and October. Maximum egg production was in April and May. Davies (1967) examined *Leuciscus leuciscus* and *R. rutilus* from the River Lugg, Herefordshire. *R. rutilus* were infected most, but not all months. The

incidence was below 10% throughout. On *L. leuciscus* high incidence occurred and worm pairs were found each month. The incidence was lowest in January (30%) and highest in May (75%), but above 50% in most months. Eggs were found from February to November, with maximum occurrence during May (100%) and June (89%).

*Discocotyle sagittata* (Leuckart, 1842)

Robertson (1953) studied this species on the gills of *Salmo trutta* at Dunalastair Reservoir, Scotland, during the months March to September. Maximum incidence was in June when 27% of the fish were infected with an average of 5·7 worms per fish. The incidence fell steadily from June to September. A sample of *S. trutta* in November showed only 9% to be infected.

Paling (1965) investigated the population dynamics of *D. sagittata* on *S. trutta* and *Salvelinus alpinus* in the lakes of Windermere and Crummock Water, England. In Windermere *S. trutta* were infected and eggs were present in the uterus of the worms during all months of the year. The maximum incidence of juvenile worms, 11·2%, was observed in July and August, and Paling showed that the infection of *S. trutta* occurred from March to November. The life span of *D. sagittata* in Windermere was established as at least three, but not more than four years. After infection of the host, described by Paling (1969), *D. sagittata* rapidly completed development of the remaining three of the four pairs of adhesive clamps. At this time, about two months post-infection, the worm had the full adult complement of clamps and sexual organs. Paling (1965) studied growth as units, an index of parasite age expressed by length × breadth measurements of the worms. During their first year of life the worms grew at least seven units, perhaps ten units. 46·5% of the population were within this size group. The worms reproduced during each successive year of life, and Paling demonstrated that the majority of the older parasites appeared to die during the warmer months of the year. In Windermere the temperature at 15 m depth was 15°C, hence the incubation time for the eggs was short, and the infection of fish reached its peak. At winter temperatures, about 5°C, the eggs present probably remained dormant, to hatch in the spring as water temperatures increased.

At Crummock Water, on *Salmo trutta* and *Salvelinus alpinus*, Paling found both species of fish to be infected during all months. However, in this lake, the life span of the parasite was demonstrated to be one year only. The maximum percentage of juvenile worms was found on *S. trutta* in September–October, 66·3%, but on *S. alpinus* in November–December, 53%. Paling concluded that there were two races of *Discocotyle*, having different life spans and breeding times, one on *S. trutta* in Windermere, the other on *S. trutta* and *S. alpinus* in Crummock Water.

Campbell (1974) studied the incidence of *D. sagittata* on *S. trutta* in Loch Leven, Scotland, from 1967 to 1972. During these years, *D. sagittata* was present on the fish throughout, but the incidence varied considerably. From June 1967 to November 1968 it increased from 35% to 80%. By April 1969 it had decreased to 20% and remained generally low until early 1970. It increased during 1970, to around 60% in June and October, but then declined again to 30%, where it

remained until the end of the survey in 1972. It is evident that the population of *D. sagittata* varied in size considerably from year to year. In Loch Leven Campbell found that the main reproductive period of *D. sagittata* was in agreement with that found by Paling (1965) in Crummock Water. Maximum incidence of juveniles was in November–December (41 %), minimum in July–August (3·5 %), although they were present in all months.

In Lake Ladoga, U.S.S.R., Bauer and Nikol'skaya (1957) examined the occurrence of *D. sagittata* on *Coregonus lavaretus baeri* natio *ladogae* from July to November. The incidence rose from 45 % in July to 90 % in September, to fall again to 47 % by October. Large worms with eggs were present in July, and increased in August, when eggs appeared on the gills. In September juvenile *D. sagittata* were present together with adult worms. The number of adults fell in October and November. The increase in incidence in September was owing to the concurrent infections of juvenile and adult worms. The adults were lost by October–November.

## III. Seasonal Studies in World Climatic Zones

The distributions of plants and animals are influenced by climate. Climate is determined by and varies with latitude, longitude and altitude. It is the end product of infinite and changing combinations of temperature, wind, rain, water currents, land and water masses, mountain ranges and vegetational cover. Climate affects parasites, not only through the host, but also directly with respect to temperature. Temperature is the most important single extrinsic factor that influences parasites (Noble and Noble, 1971). With some species of monogeneans, temperature tolerances and the effect of temperature on development have been studied, but the number for which this information is available is small. Although temperature may ultimately be the most relevant factor for understanding the world-wide occurrence and seasonal dynamics of the monogeneans, the information currently available is too fragmentary to attempt such an analysis. As a less satisfactory, but hopefully viable alternative, the seasonal studies reported in Section II are related to the major climatic regions of the world (Fig. 1). At least this analysis provides a clear demonstration of the regions of the world where no seasonal studies of monogeneans have been carried out.

The climate zones shown on the map (Fig. 1) are divided into 1. tropical, 2. subtropical, 3. mid-latitude or temperate, 4. polar and 5. mountain climates. The tropical climates (1) are grouped on both sides of the equator. The rainy tropical climate (1a), with ample year-long moisture, is found in relatively small zones in South America, Africa and coastal regions of India and Indonesia. In the other humid tropical climates, savanna (1b) and highland (1c), the rainfall is seasonal. In the tropical semi-deserts (1d) and deserts (1e) there may be no regular wet season. Such arid areas also have great diurnal extremes of temperature.

The subtropical climates include the Mediterranean type (2a), characterised by hot dry summers and warm wet winters, and found on the western coasts of

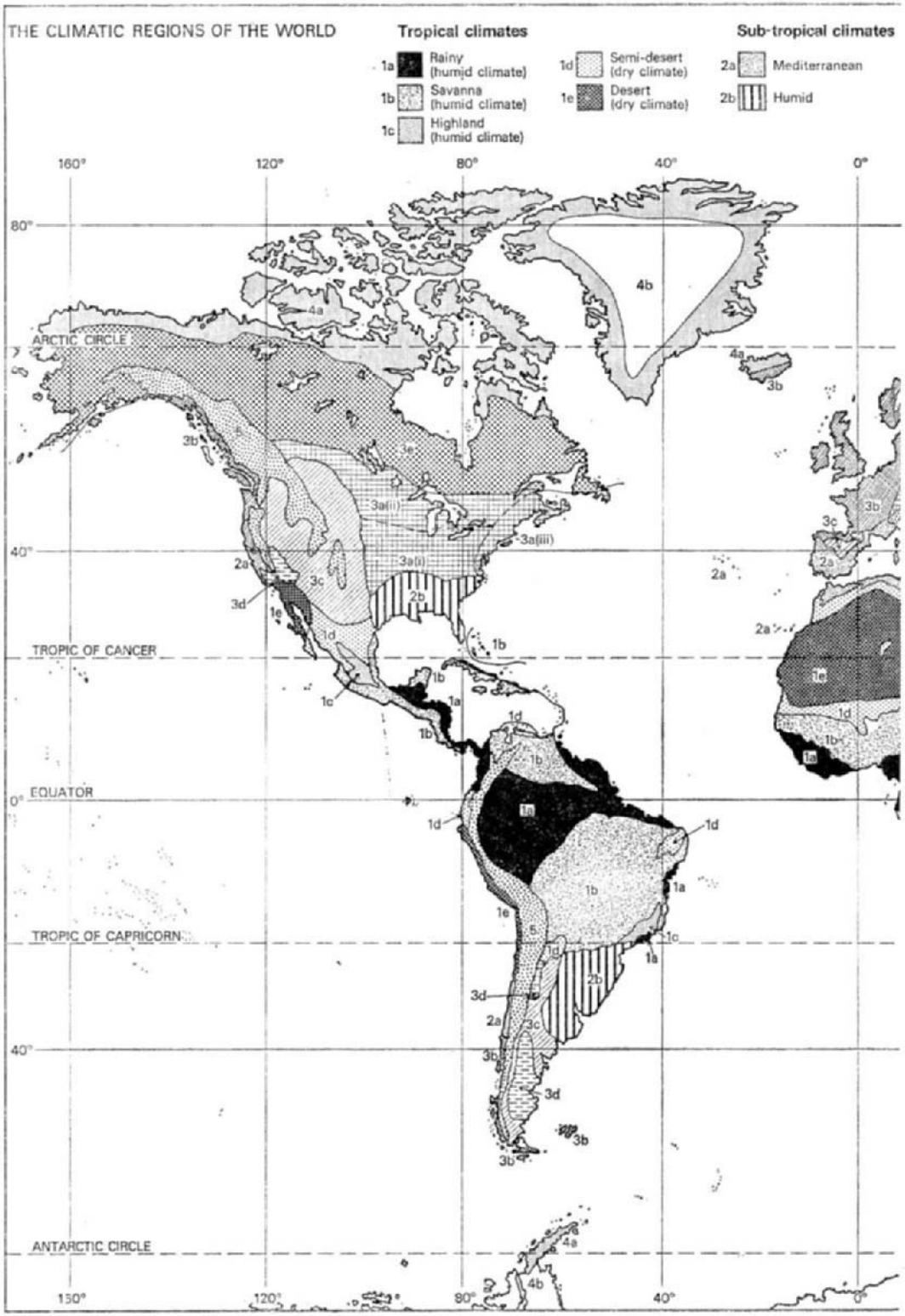
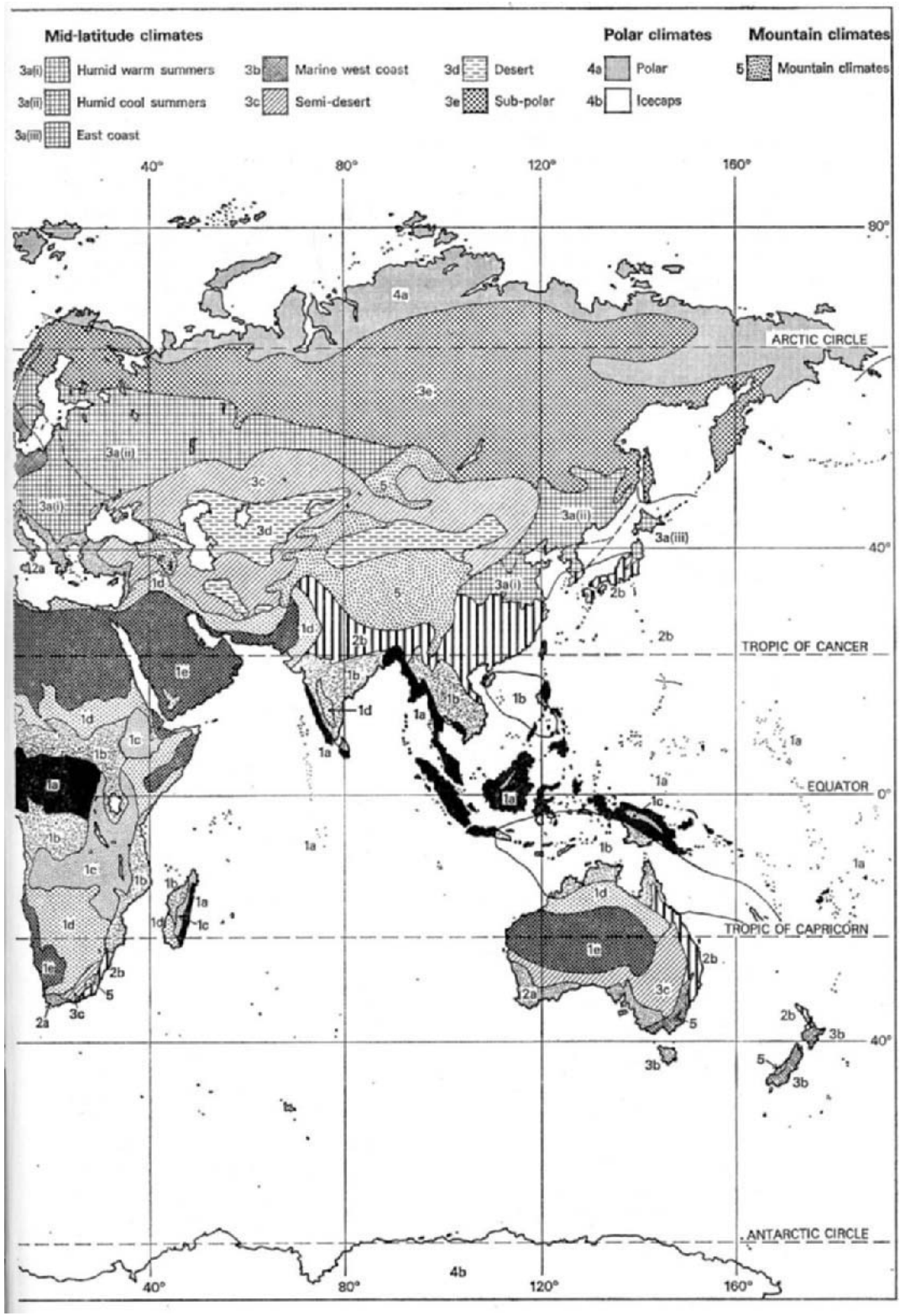



FIG. 1. The climatic regions of the world. The information contained in this map is based on that used
Heinemann, London (1973) and is reproduced




in "New Relief World Atlas": Paul Hamlyn, London (1966) and Edlin, H. L. "Atlas of Plant Life": by permission of Geographical Projects, London.

the continents. On the eastern coasts the humid subtropics (2b) occur. These have heavier, but still seasonal, rainfall.

In all the mid-latitude or temperate climates the winters are cold enough so that plant growth has a characteristic resting period. The humid zones (3a) are divided into those with warm summers (3a i) and those with cool summers (3a ii). A further zone (3a iii) occurs on the east coasts of the continents. On the west coasts of the continents a marine west coast climate (3b) occurs. Inland, in the drier central zones of the larger land masses, cool semi-desert (3c) and desert (3d) areas are found, as in Central Asia, inland California, Argentina and Australia. A sub-polar climatic zone (3e) runs across Asia, Europe and North America. The summer is so short in this zone that only a limited fauna and flora can exist.

Further to the north the polar climate (4a) bordering the Arctic Ocean has a very brief summer. This tundra area has the ground continually frozen just below the surface. The Greenland and Antarctic ice-caps (4b) are permanently frozen regions.

The mountain climates (5) break the continuous, although irregular, distribution of the other climatic zones north and south of the equator.

## A. TROPICAL (Climate zone 1)

No seasonal studies on monogenean parasites from any of the tropical climate zones are known to the author.

## B. SUBTROPICAL (Climate zone 2)

### 1. *Mediterranean* (Climate zone 2a)

Four species have been studied (Table I). In *Actinocleidus fergusoni* and *Urocleidus ferox* reproduction continued throughout the year in Sacramento County, California (Crane and Mizelle, 1968). Population peaks occurred during January, May and July for *A. fergusoni* and in January, April and August for *U. ferox*. In *A. fergusoni* the peaks had no consistent relationship with temperature since they occurred at 8°C, 25°C and 27°C. With *U. ferox* the peaks for April and August showed a positive relationship with high temperatures 22°C and 28°C, but the peak in January occurred at a time when the annual temperature was low (8°C). The life span of both species was not known, but the continuous presence of worms was not entirely attributable to adult worms surviving for long periods, as developmental stages occurred all year.

*Dactylogyrus vastator* was studied in Israel by Paperna (1963). There was a marked seasonal cycle of occurrence, from April to June. This was related to temperature, as at temperatures below 12°C and above 37°C oviposition ceased. The optimum temperature for rate of egg development was 28°C. The life span was believed to be about 3–5 days. In Israel *D. vastator* was found on *Cyprinus carpio* up to 60 mm long, and not above, and Paperna postulated that survival from one year to the next must be by a diapause at the egg stage. The eggs hatch only upon the appearance of fry in the spring. It was necessary to postulate a diapause owing to the fact that the winter water temperature in

Israel seldom drops below 10°C, and at this temperature eggs continued to develop to hatch in 15 days. Without a diapause, the persistent infection of *D. vastator* in Israel could not be explained, although the existence of a diapause has been questioned by most other workers (Paperna, 1963). The occurrence of *D. vastator* in Israel was studied in fish farms and the laboratory and may accordingly be atypical when compared with natural populations. Furthermore *D. vastator* was introduced into Israel with European *Cyprinus carpio*; it is not a natural resident of the area, neither are *D. anchoratus*, *D. extensus* nor *D. minutus* which also appeared on *C. carpio* in Israel (Paperna, 1964a,b).

2. *Humid* (Climate zone 2b)

In this climate zone Rawson and Rogers (1972a,b, 1973) studied the monogeneans infecting *Lepomis macrochirus* and *Micropterus salmoides*. In summary, their species on *L. macrochirus* were divided into Group 1, large species most abundant during autumn and least abundant during the mid-summer period (*Anchoradiscus triangularis*, *Clavunculus bifurcatus*), Group 2, large species most abundant during the spring (*Cleidodiscus nematocirrus*, *C. robustus*, *Lyrodiscus seminolensis*) and Group 3, small species abundant during the summer but with a prewinter peak at water temperatures near 10°C (*Actinocleidus fergusoni*, *Urocleidus acer*, *U. dispar*, *U. ferox*). The largest species (Groups 1 and 2) reached maximum abundance during the cool spring and autumn months, whilst the small species (Group 3) were abundant during the summer at temperatures above 26°C. At 26°C the larger species were extremely rare, and almost completely absent above 30°C. The summer increase of the small species coincided with temperatures at which *L. macrochirus* spawning was maximum, and the autumn decline of the parasites coincided with a fall in temperature below this range. *L. macrochirus* congregated for spawning, which behaviour probably contributed to the increased parasite numbers (Rawson and Rogers, 1972b).

*Gyrodactylus macrochiri* was studied on *Lepomis macrochirus* and also on *Micropterus salmoides*. Population levels were high under three circumstances during the spring reproductive period of the fish when close association and stress of spawning created conditions favourable to the parasite infections, during the autumn, when temperatures were similar to spawning temperatures, and during the winter when temperatures were below 10°C (Rawson and Rogers, 1973).

The other four monogeneans of *Micropterus salmoides*, *Actinocleidus fusiformis*, *Clavunculus bursatus*, *Urocleidus furcatus* and *U. principalis* also had population trends which seemed to be associated with water temperature. *U. furcatus* and *U. principalis* were at moderate to low levels of occurrence during winter. *C. bursatus* and *A. fusiformis* had peaks prior to the spring period of maximum temperature increase. A fall in abundance during this period of maximum water temperature increase was seen in all four species, but as the temperature rose to 28°C in late June, and also fell to 28°C in mid-September, the abundance of all species except *C. bursatus* peaked, which suggested a temperature optimum. During mid-summer, with surface water temperature at 30°C and above, the three species remained at a moderately high level. After

TABLE I

*Studies on seasonal occurrence of monogeneans listed in the climate zones of the World (see Fig. 1). (The species are in alphabetical order.)*

| Climate zones | Monogenean species | Host species | Locality | Authors |
|---|---|---|---|---|
| 1. **Tropical** | | | | |
| 1a. RAINY (humid climate) | no seasonal studies | | tropical forest | |
| 1b. SAVANNA (humid climate) | no seasonal studies | | tropical grassland | |
| 1c. HIGHLAND (humid climate) | no seasonal studies | | tropical highland | |
| 1d. SEMI-DESERT (dry climate) | no seasonal studies | | hot semi-desert | |
| 1e. DESERT (dry climate) | no seasonal studies | | hot desert | |
| 2. **Subtropical** | | | | |
| 2a. MEDITERRANEAN | | | scrub, woodland, olive | |
| | *Actinocleidus fergusoni* | *Lepomis macrochirus* | Sacramento Co., Calif., U.S.A. | Crane and Mizelle (1968) |
| | *Dactylogyrus extensus* | *Cyprinus carpio* | Israel | Paperna (1964a) |
| | *Dactylogyrus vastator* | *Cyprinus carpio* | Israel | Paperna (1963) |
| | *Urocleidus ferox* | *Lepomis macrochirus* | Sacramento Co., Calif., U.S.A. | Crane and Mizelle (1968) |
| 2b. HUMID | | | deciduous forest | |
| | *Actinocleidus fergusoni* | *Lepomis macrochirus* | Walter F. George Res., Alabama, U.S.A. | Rawson and Rogers (1972b) |
| | *Actinocleidus fusiformis* | *Micropterus salmoides* | Walter F. George Res. Alabama, U.S.A. | Rawson and Rogers (1972a) |
| | *Anchoradiscus triangularis* | *Lepomis macrochirus* | Walter F. George Res. Alabama, U.S.A. | Rawson and Rogers (1972b) |

| | | | | |
|---|---|---|---|---|
| | *Clavunculus bifurcatus* | *Lepomis macrochirus* | Walter F. George Res., Alabama, U.S.A. | Rawson and Rogers (1972b) |
| | *Clavunculus bursatus* | *Micropterus salmoides* | Walter F. George Res., Alabama, U.S.A. | Rawson and Rogers (1972a) |
| | *Cleidodiscus nematocirrus* | *Lepomis macrochirus* | Walter F. George Res., Alabama, U.S.A. | Rawson and Rogers (1972b) |
| | *Cleidodiscus robustus* | *Lepomis macrochirus* | Walter F. George Res., Alabama, U.S.A. | Rawson and Rogers (1972b) |
| | *Diplozoon nipponicum* | *Cyprinus carpio* | Tokyo, Japan | Kamegai (1970) |
| | *Gyrodactylus macrochiri* | *Lepomis macrochirus*; *Micropterus salmoides* | Walter F. George Res., Alabama, U.S.A. | Rawson and Rogers (1973) |
| | *Lyrodiscus seminolensis* | *Lepomis macrochirus* | Walter F. George Res., Alabama, U.S.A. | Rawson and Rogers (1972b) |
| | *Urocleidus acer* | *Lepomis macrochirus* | Walter F. George Res., Alabama, U.S.A. | Rawson and Rogers (1972b) |
| | *Urocleidus dispar* | *Lepomis macrochirus* | Walter F. George Res., Alabama, U.S.A. | Rawson and Rogers (1972b) |
| | *Urocleidus ferox* | *Lepomis macrochirus* | Walter F. George Res., Alabama, U.S.A. | Rawson and Rogers (1972b) |
| | *Urocleidus furcatus* | *Micropterus salmoides* | Walter F. George Res., Alabama, U.S.A. | Rawson and Rogers (1972a) |
| | *Urocleidus principalis* | *Micropterus salmoides* | Walter F. George Res., Alabama, U.S.A. | Rawson and Rogers (1972a) |
| **3. Mid-latitude** | | | | |
| 3a i. HUMID WARM SUMMERS | | | temperate grassland, mixed forest | |
| | *Dactylogyrus anchoratus* | *Cyprinus carpio* | Opole Lubelskie, Poland | Prost (1963) |
| | *Dactylogyrus borealis** | *Phoxinus phoxinus* | Balaton, Hungary | Molnár (1968) |

* Molnár did not separate the individuals of each species of *Dactylogyrus* and *Gyrodactylus*.

TABLE 1—*continued*

| Climate zones | Monogenean species | Host species | Locality | Authors |
|---|---|---|---|---|
| 3a i—*continued* | | | | |
| | *Dactylogyrus extensus* | *Cyprinus carpio* | Opole Lubelskie, Poland | Prost (1963) |
| | *Dactylogyrus lamellatus* | *Ctenopharyngodon idella* | Szarvas and Dinnyés fish farms, Hungary | Molnár (1971a,b) |
| | *Dactylogyrus merus** | *Phoxinus phoxinus* | Balaton, Hungary | Molnár (1968) |
| | *Dactylogyrus phoxini** | *Phoxinus phoxinus* | Balaton, Hungary | Molnár (1968) |
| | *Dactylogyrus vastator* | *Cyprinus carpio* | Germany | Wunder (1929) |
| | *Gyrodactylus aphyae** | *Phoxinus phoxinus* | Balaton, Hungary | Molnár (1968) |
| | *Gyrodactylus laevis** | *Phoxinus phoxinus* | Balaton, Hungary | Molnár (1968) |
| | *Gyrodactylus macronychus*[a] | *Phoxinus phoxinus* | Balaton, Hungary | Molnár (1968) |
| | *Gyrodactylus phoxini** | *Phoxinus phoxinus* | Balaton, Hungary | Molnár (1968) |
| | *Pseudacolpenteron pavlovskii* | *Cyprinus carpio* | Bulgaria | Margaritov (1965) |
| 3a ii. HUMID COOL SUMMERS | | | temperate grassland, mixed forest | |
| | *Ancyrocephalus paradoxus* | *Lucioperca lucioperca*; *Perca fluviatilis* | Rybinsk Res., U.S.S.R. | Izyumova (1958b) |
| | *Ancyrocephalus percae* | *Perca fluviatilis* | Lake Dusia, Lithuanian S.S.R. | Rautskis (1970a) |
| | *Dactylogyrus alatus* | *Blicca bjoerkna* | Rybinsk Res. U.S.S.R. | Izyumova (1960, 1964) |
| | *Dactylogyrus amphibothrium* | *Gymnocephalus cernua* | Rybinsk Res., U.S.S.R. | Izyumova (1960, 1964) |
| | *Dactylogyrus aristichthys* | *Aristichthys nobilis* | Moscow area, U.S.S.R. | Bauer *et al.* (1969) |
| | *Dactylogyrus auriculatus* | *Abramis brama* | Rybinsk Res., U.S.S.R. | Izyumova (1960, 1964) |

| | | | |
|---|---|---|---|
| *Dactylogyrus chranilowi* | *Abramis ballerus* | Rybinsk Res., U.S.S.R. | Izyumova (1960, 1964) |
| *Dactylogyrus cornu* | *Blicca bjoerkna*; *Rutilus rutilus* | Rybinsk Res., U.S.S.R. | Izyumova (1960) |
| *Dactylogyrus crucifer* | *Rutilus rutilus* | Rybinsk Res., U.S.S.R. | Izyumova (1960) |
| *Dactylogyrus extensus* | *Cyprinus carpio* | Ukrainian S.S.R. | Ivasik (1953) |
| *Dactylogyrus falcatus* | *Abramis brama* | River Volga, U.S.S.R. | Bogdanova (1958) |
| | *Abramis brama* | Rybinsk Res., U.S.S.R. | Izyumova (1960, 1964) |
| *Dactylogyrus fallax* | *Rutilus rutilus* | Rybinsk Res., U.S.S.R. | Izyumova (1960, 1964) |
| *Dactylogyrus iwanowi* | *Leuciscus brandti* | Peter the Great Bay, nr Vladivostok, U.S.S.R. | Bykhovskii (1957) |
| *Dactylogyrus lamellatus* | *Ctenopharyngodon idella* | No specific locality | Bauer *et al.* (1969) |
| *Dactylogyrus macracanthus* | *Tinca tinca* | River Donets, U.S.S.R. | Komarova (1957) |
| *Dactylogyrus minor* | *Rutilus rutilus* | Rybinsk Res., U.S.S.R. | Izyumova (1960) |
| *Dactylogyrus nanus* | *Abramis brama*; *Blicca bjoerkna*; *Rutilus rutilus* | Rybinsk Res., U.S.S.R. | Izyumova (1960, 1964) |
| *Dactylogyrus nasalis* | *Rutilus rutilus* | Vrevo Lake, nr Leningrad, U.S.S.R. | Strelkov *et al.* (1969) |
| *Dactylogyrus nobilis* | *Aristichthys nobilis* | European U.S.S.R. | Bauer *et al.* (1969) |
| *Dactylogyrus similis* | *Blicca bjoerkna*; *Rutilus rutilus* | Rybinsk Res., U.S.S.R. | Izyumova (1960) |
| *Dactylogyrus simplicimalleata* | *Abramis brama*; *Pelecus cultratus* | Rybinsk Res., U.S.S.R. | Izyumova (1958b, 1960) |
| *Dactylogyrus sphyrna* | *Rutilus rutilus* | Rybinsk Res., U.S.S.R. | Izyumova (1960) |
| *Dactylogyrus vastator* | *Cyprinus carpio* | Various localities | Summarized by Bykhovskii (1957; Bauer (1959a); Bauer *et al.* (1969) |

* Molnár did not separate the individuals of each species of *Dactylogyrus* and *Gyrodactylus*.

TABLE 1—*continued*

| Climate zones | Monogenean species | Host species | Locality | Authors |
|---|---|---|---|---|
| 3a ii—*continued* | | | | |
| | *Dactylogyrus wunderi* | *Abramis brama*; *Blicca bjoerkna* | Rybinsk Res., U.S.S.R. | Izyumova (1960, 1964) |
| | *Dactylogyrus zandti* | *Abramis brama* | River Volga, U.S.S.R. | Bogdanova (1958) |
| | *Diplozoon paradoxum* | *Abramis brama*; *Blicca bjoerkna*; *Rutilus rutilus* | Rybinsk Res., U.S.S.R. | Izyumova (1960, 1964) |
| | *Discocotyle sagittata* | *Coregonus lavaretus baeri* natio *ladogensis* | Lake Ladoga, U.S.S.R. | Bauer and Nikol'skaya (1957) |
| | *Gyrodactylus arcuatus* Bykhovskii, 1933 | *Gasterosteus aculeatus*; *Pungitius pungitius* | Neva delta Res. U.S.S.R. | Banina and Isakov (1972) |
| | *Gyrodactylus arcuatus*† Bykhovskii *sensu* Bykhovskii & Polyanski, 1953 | *Gasterosteus aculeatus* | Nämdo, Baltic Sea, Sweden | Malberg (1970) |
| | *Gyrodactylus bychowskyi*† | *Gasterosteus aculeatus*; *Pungitius pungitius* | Neva delta Res., U.S.S.R. | Banina and Isakov (1972) |
| | *Gyrodactylus lucii* | *Esox lucius* | Lake Dusia, Lithuanian S.S.R. | Rautskis (1970b) |
| | *Gyrodactylus pungitii* | *Pungitius pungitius* | Sweden | Malmberg (1970) |
| | *Gyrodactylus rarus* | *Gasterosteus aculeatus*; *Pungitius pungitius* | Neva delta Res., U.S.S.R. | Banina and Isakov (1972) |
| | | *Pungitius pungitius* | Sweden | Malmberg (1970) |
| | *Tetraonchus monenteron* | *Esox lucius* | River Oka, U.S.S.R. | Markova (1958) |
| | | | Rybinsk Res., U.S.S.R. | Izyumova (1960, 1964) |
| | | | Lake Dusia, Lithuanian S.S.R. | Rautskis (1970b) |
| | *Urocleidus adspectus* | *Perca flavescens* | Bay of Quinte, Lake Ontario, Canada | Tedla and Fernando (1969) |

| | | | | | |
|---|---|---|---|---|---|
| 3a iii. | EAST COAST | | no seasonal studies | temperate grassland, mixed forest | |
| 3b. | MARINE WEST COAST | | | temperate grassland, deciduous forest | |
| | | *Ancyrocephalus paradoxus* | *Perca fluviatilis* | Lake Dargin, Poland | Wierzbicki (1970) |
| | | *Dactylogyrus amphibothrium* | *Gymnocephalus cernua* | Hanningfield Res., Essex, England | Wootten (pers. comm.) |
| | | *Dactylogyrus auriculatus* | *Abramis brama* | Lake Dabie, Poland | Wierzbicka (1974) |
| | | *Dactylogyrus chranilowi* | *Abramis ballerus* | Lake Dabie, Poland | Wierzbicka (1974) |
| | | *Dactylogyrus cordus* | *Leuciscus leuciscus* | River Lugg, Herefordshire, England | Davies (1967) |
| | | *Dactylogyrus cornoides* | *Blicca bjoerkna* | Lake Dabie, Poland | Wierzbicka (1974) |
| | | *Dactylogyrus cornu* | *Blicca bjoerkna* | Lake Dabie, Poland | Wierzbicka (1974) |
| | | *Dactylogyrus crucifer* | *Rutilus rutilus* | Rostherne Mere, Cheshire, England; | Rizvi (1964, 1969) |
| | | | | Shropshire Union Canal, Cheshire, England; | Mishra (1966) |
| | | | | River Lugg, Herefordshire, England | Davies (1967) |
| | | *Dactylogyrus distinguendus* | *Blicca bjoerkna* | Lake Dabie, Poland | Wierzbicka (1974) |
| | | *Dactylogyrus falcatus* | *Abramis brama* | Lake Dabie, Poland | Wierzbicka (1974) |
| | | *Dactylogyrus fallax* | *Blicca bjoerkna* | Lake Dabie, Poland | Wierzbicka (1974) |
| | | *Dactylogyrus macracanthus* | *Tinca tinca* | Germany | Wilde (1935, 1937) |
| | | *Dactylogyrus prostae* | *Leuciscus squalius* | River Lugg, Herefordshire, England | Davies (1967) |

† *Gyrodactylus arcuatus* Bykhovskii *sensu* Bykhovskii & Polyanski, 1953 was regarded as a separate species from *G. arcuatus* Bykhovskii, 1933 by Malmberg, 1970. Malmberg also considered *G. bychowskyi* as a synonym of *G. arcuatus* Bykhovskii *sensu* Bykhovskii & Polyanski, 1953.

TABLE 1—*continued*

| Climate zones | Monogenean species | Host species | Locality | Authors |
|---|---|---|---|---|
| 3b—*continued* | | | | |
| | *Dactylogyrus similis* | *Rutilus rutilus* | Rostherne Mere, Cheshire, England | Rizvi (1964, 1969) |
| | *Dactylogyrus sphyrna* | *Blicca bjoerkna* | Lake Dabie, Poland | Wierzbicka (1974) |
| | | *Rutilus rutilus* | Shropshire Union Canal, Cheshire, England | Mishra (1966) |
| | *Dactylogyrus vastator* | *Cyprinus carpio* | Sweden | Nordquist (1925); Nybelin (1925) |
| | *Dactylogyrus vistulae* | *Leuciscus cephalus* | River Lugg, Herefordshire, England | Davies (1967) |
| | *Dactylogyrus wunderi* | *Abramis brama* | Shropshire Union Canal, Cheshire, England | Mishra (1966) |
| | | | Lake Dabie, Poland | Wierzbicka (1974) |
| | *Dactylogyrus zandti* | *Abramis brama* | Lake Dabie, Poland | Wierzbicka (1974) |
| | *Diplozoon gussevi* | *Blicca bjoerkna* | Lake Dabie, Poland | Wierzbicka (1974) |
| | *Diplozoon nagibinae* | *Abramis ballerus* | Lake Dabie, Poland | Wierzbicka (1974) |
| | *Diplozoon paradoxum* | *Abramis brama* | Lakes Neuchâtel, Bienne, Morat, Switzerland; | Bovet (1967) |
| | | | River Glomma, Norway; | Halvorsen (1972) |
| | | | Shropshire Union Canal, Cheshire, England; | Mishra (1966) |
| | | | Lake Dabie, Poland | Wierzbicka (1974) |
| | | *Abramis brama* x *Rutilus rutilus* hybrids | River Glomma, Norway | Halvorsen (1972) |

| | | | |
|---|---|---|---|
| | *Blicca bjoerkna* | Lakes Neuchâtel, Bienne, Morat, Switzerland | Bovet (1967) |
| | *Leuciscus leuciscus* | River Lugg, Herefordshire, England | Davies (1967) |
| | *Phoxinus phoxinus* | Germany | Zeller (1872) |
| | *Rutilus rutilus* | Shropshire Union Canal, Cheshire, England; | Mishra (1966) |
| | | Lakes Neuchâtel, Bienne, Morat, Switzerland; | Bovet (1967) |
| | | River Glomma, Norway | Halvorsen (1972) |
| *Discocotyle sagittata* | *Salmo trutta* | Lakes Crummock Water and Windermere, England; | Paling (1965) |
| | | Loch Leven, Scotland | Campbell (1974) |
| | *Salvelinus alpinus* | Crummock Water, England | Paling (1965) |
| *Gyrodactylus alexanderi* | *Gasterosteus aculeatus* | British Columbia, Canada | Lester (1974) |
| *Gyrodactylus anguillae* | *Anguilla anguilla* (elvers) | River Morup, Falkenberg, Halland, Sweden | Malmberg (1970) |
| *Gyrodactylus elegans* | *Abramis ballerus*<br>*Abramis brama*<br>*Blicca bjoerkna* | Lake Dabie, Poland | Wierzbicka (1974) |
| *Gyrodactylus laevis* | *Abramis ballerus*<br>*Blicca bjoerkna* | Lake Dabie, Poland | Wierzbicka (1974) |
| *Gyrodactylus rarus* | *Gasterosteus aculeatus* | Pond, Baildon Moor, Yorkshire, England | Chappell (1969) |

TABLE 1—*continued*

| Climate zones | Monogenean species | Host species | Locality | Authors |
|---|---|---|---|---|
| 3b.—*continued* | | | | |
| | *Tetraonchus borealis* | *Thymallus thymallus* | River Lugg, Herefordshire, England | Davies (1967) |
| | *Tetraonchus monenteron* | *Esox lucius* | Llyn Tegid, Wales; | Chubb (1961) |
| | | | Rostherne, Mere, Cheshire, England; | Rizvi (1964, 1969) |
| | | | Shropshire Union Canal, Cheshire, England; | Mishra (1966) |
| | | | River Lugg, Herefordshire, England | Davies (1967) |
| 3c. SEMI-DESERT | | | prairie and steppe | |
| | *Ancyrocephalus paradoxus* | *Lucioperca lucioperca* | Dnepr Delta, U.S.S.R. | Komarova (1964) |
| | *Dactylogyrus aristichthys* | *Aristichthys nobilis* | Krasnodar Territory, U.S.S.R. | Bauer *et al.* (1969) |
| | *Dactylogyrus auriculatus* | *Abramis brama* | Dnepr Delta, U.S.S.R. | Komarova (1964) |
| | *Dactylogyrus cabolleroi* | *Rutilus rutilus* | Iriklin Res., River Ural, U.S.S.R. | Kashkovski (1967) |
| | *Dactylogyrus cornu* | *Blicca bjoerkna*; *Vimba vimba vimba* natio *carinata* | Dnepr Delta, U.S.S.R. | Komarova (1964) |
| | | *Rutilus rutilus* | Iriklin Res. River Ural, U.S.S.R. | Kashkovski (1967) |
| | *Dactylogyrus crucifer* | *Rutilus rutilus* | Iriklin Res., River Ural, U.S.S.R. | Kashkovski (1967) |
| | | *R. rutilus heckeli* | Dnepr Delta, U.S.S.R. | Komarova (1964) |

| | | | |
|---|---|---|---|
| *Dactylogyrus falcatus* | *Abramis brama* | Dnepr Delta, U.S.S.R. | Komarova (1964) |
| | *Rutilus rutilus* | Iriklin Res., River Ural, U.S.S.R. | Kashkovski (1967) |
| *Dactylogyrus fallax* | *Rutilus rutilus* | Iriklin Res., River Ural, U.S.S.R. | Kashkovski (1967) |
| *Dactylogyrus haplogonus* | *Vimba vimba vimba* natio *carinata* | Dnepr Delta, U.S.S.R. | Komarova (1964) |
| *Dactylogyrus hypophthalmichthys* | *Hypophthalmichthys molitrix* | Krasnodar Territory U.S.S.R. | Bauer *et al.* (1969) |
| *Dactylogyrus nanus* | *Rutilus rutilus* | Iriklin Res., River Ural, U.S.S.R. | Kashkovski (1967) |
| *Dactylogyrus ramulosus* | *Rutilus rutilus* | Iriklin Res., River Ural, U.S.S.R. | Kashkovski (1967) |
| *Dactylogyrus similis* | *Blicca bjoerkna* | Dnepr Delta, U.S.S.R. | Komarova (1964) |
| | *Rutilus rutilus* | Iriklin Res., River Ural, U.S.S.R. | Kashkovski (1967) |
| *Dactylogyrus simplicimalleata* | *Pelecus cultratus* | Dnepr Delta, U.S.S.R. | Komarova (1964) |
| *Dactylogyrus sphyrna* | *Blicca bjoerkna*; *Rutilus rutilus heckeli*; *Vimba vimba vimba* natio *carinata* | Dnepr Delta, U.S.S.R. | Komarova (1964) |
| | *Rutilus rutilus* | Iriklin Res., River Ural, U.S.S.R. | Kashkovski (1967) |
| *Dactylogyrus wunderi* | *Abramis brama* | Dnepr Delta, U.S.S.R. | Komarova (1964) |
| *Dactylogyrus zandti* | *Abramis brama* | Dnepr Delta, U.S.S.R. | Komarova (1964) |
| *Diplozoon bliccae* | *Blicca bjoerkna* | Dnepr Delta, U.S.S.R. | Komarova (1964) |
| *Diplozoon homoion* | *Rutilus rutilus* | Iriklin Res., River Ural, U.S.S.R. | Kashkovski (1967) |
| *Diplozoon markewitschi* | *Vimba vimba vimba* natio *carinata* | Dnepr Delta, U.S.S.R. | Komarova (1964) |
| *Diplozoon paradoxum* | *Abrama brama* *Rutilus rutilus heckeli* | Dnepr Delta, U.S.S.R. | Komarova (1964) |

TABLE 1—*continued*

| Climate zones | Monogenean species | Host species | Locality | Authors |
|---|---|---|---|---|
| 3c.—*continued* | | | | |
| | *Gyrodactylus parvicopula* | *Rutilus rutilus* | Iriklin Res., River Ural, U.S.S.R. | Kashkovski (1967) |
| | *Tetraonchus monenteron* | *Esox lucius* | Dnepr Delta, U.S.S.R. | Komarova (1964) |
| 3d. DESERT | no seasonal studies | | cool desert | |
| 3e. SUB-POLAR | | | coniferous forest | |
| | *Dactylogyrus crucifer* | *Rutilus rutilus* | Lake Srednego Kuĭto, Karelia, U.S.S.R. | Rumyantsev (1972) |
| | | | Karelian lakes, U.S.S.R. | Shul'man *et al.* (1974) |
| | *Dactylogyrus fallax* | *Rutilus rutilus* | Lake Srednego Kuĭto, Karelia, U.S.S.R. | Rumyantsev (1972) |
| | *Dactylogyrus nanus* | *Rutilus rutilus* | Lake Srednego Kuĭto, Karelia, U.S.S.R. | Ruymyantsev (1972) |
| | | | Karelian lakes, U.S.S.R. | Shul'man *et al.* (1974) |
| | *Dactylogyrus similis* | *Rutilus rutilus* | Lake Srednego Kuĭto, Karelia, U.S.S.R. | Rumyantsev (1972) |
| | *Tetraonchus monenteron* | *Esox lucius* | Karelian lakes, U.S.S.R. | Shul'man *et al.* (1974) |
| **4. Polar** | | | | |
| 4a. POLAR | no suitable habitats for freshwater monogeneans | | tundra | |
| 4b. ICE-CAPS | no suitable habitats for freshwater monogeneans | | icefields and glaciers | |
| **5. Mountain** | no seasonal studies | | heath, rocks and scree | |

the autumn fall in abundance noted above, the populations of all four species peaked to extremely high levels as the temperature approached 9°C, thereafter the populations declined (Rawson and Rogers, 1972a). Such an event was found by Crane and Mizelle (1968) with *Actinocleidus fergusoni* and *Urocleidus ferox* in Sacramento County (see Section IIIB1).

Rawson and Rogers (1972a,b) speculated that the high population levels of these monogeneans at about 10°C may have involved a reduction in the immune response of the fish, owing to the cessation of antibody production. Avtalion *et al.* (1973) have reviewed the effect of environmental temperature on the immune response of fish, and antibody response was dependent upon temperature, normally having more effect at higher temperatures. However, the effects of fish antibody on freshwater monogeneans have not been studied experimentally.

Kamegai (1970) studied gonad development of *Diplozoon nipponicum*. The material was collected during 2 years, from a number of localities which were not specified. It is assumed that the material originated in climate zone 2b, but some may have come from the more northern regions of Japan, in climate zone 3a iii, mid-latitude, east coast. The study showed that the worm needed one year to develop from egg to adult, and that oviposition occurred during the summer months. The pattern of occurrence was similar to that found for other species of *Diplozoon* (Section IIIC2, 4, 5).

## C. MID-LATITUDE (Climate zone 3)

### 1. *Humid warm summers* (Climate zone 3a i)

Seasonal studies have been carried out in Poland (Prost, 1963) and Hungary (Molnár, 1968, 1971a,b). Molnár (1968) studied the collective incidence of *Dactylogyrus borealis*, *D. merus* and *D. phoxini* on *Phoxinus phoxinus*. The worms occurred from April to October. They were most abundant in June and July. No experimental details concerning their optimum temperatures are available. Molnár also studied the collective incidence of *Gyrodactylus aphyae*, *G. laevis*, *G. macronychus*, *G. phoxini* and *G.* sp. on *P. phoxinus*. These viviparous gyrodactylids occurred all year, mostly at a high level of incidence, up to 100%, with maximum infection in the spring and minimum in the autumn.

Prost (1963) investigated *Dactylogyrus anchoratus* and *D. extensus* at the Opole Lubelskie fish farm, providing much detailed information concerning the development of both species. The detailed seasonal dynamics were not given, but worms of both species occurred throughout the year. Water temperature was shown to be the main factor influencing the development of both species, at all stages in their life-cycles. No development of *D. anchoratus* eggs at 3°C occurred, but some remained viable to develop with a subsequent rise in temperature. A temperature of 22°C–23°C was optimum for embryonic development of the egg. After infection of *Cyprinus carpio*, the worm reached maturity in 6 days at 23°C–25°C and commenced oviposition. *D. extensus* eggs at 3°C also remained viable without development; 16°C–17°C was optimal for development. Development after infection of the fish took 6–7 days at 24°C–25°C. Prost was able to demonstrate clearly the important effect of tempera-

ture on all stages in the life of the worms and to show that eggs could over winter and remain viable at the bottom of the water.

Molnár (1971a,b,c, 1972) studied *Dactylogyrus lamellatus* in pond fish farm conditions. Fish of all sizes and ages were infected throughout the year but a rise in temperature increased infection levels. Temperature was again shown to control the speed of development of all stages; the oviposition rate was maximum at 28°C, but eggs were laid at temperatures down to 12°C. Hatching occurred after 1½ days at 26°C; longer times were needed above and below that temperature. Larval parasites reached maturity more quickly at higher temperatures, 8 days at 17°C–19°C, 4 days at 22°C–26°C.

2. *Humid cool summers* (Climate zone 3a ii)

This zone covers much of the European part of the U.S.S.R. where extensive study has been made of the occurrence of parasites on freshwater fishes. The findings are summarised by first reporting the occurrence of monogeneans in the natural habitat, and secondly by noting experimental observations about their development.

In the natural habitat the following species have been reported as infecting the fish throughout the year, although often with a reduced incidence and intensity of infection during the cold winter months (parentheses denote references only): *Ancyrocephalus paradoxus* (Izyumova, 1958b), *Dactylogyrus amphibothrium* (Izyumova, 1964), *D. chranilowi* (Izyumova, 1964), *D. crucifer* (Izyumova, 1960), *D. extensus* (Ivasik, 1953), *D. iwanowi* (Bykhovskii, 1957), *D. simplicimalleata* (Izyumova, 1958b), *D. sphyrna* (Izyumova, 1960), *D. vastator* (Bauer, 1959a), *Diplozoon paradoxum* (Izyumova, 1964), *Gyrodactylus arcuatus* Bykhovskii, 1933 *sensu* Bykhovskii & Polyanskii, 1953 (Malmberg, 1970), *G. pungitii* (Malmberg, 1970), *G. rarus* (Malmberg, 1970), *Urocleidus adspectus* (Tedla and Fernando, 1969). The additional species have been reported in almost all months, except those specified: *Discocotyle sagittata*, not studied December to June (Bauer and Nikol'skaya, 1957), *Gyrodactylus arcuatus* Bykhovskii, 1933, not found February and March (Banina and Isakov, 1972), *G. lucii*, not found January to March (Rautskis, 1970b), *Tetraonchus monenteron*, not found January and February (Markova, 1958). It seems likely that these four species will be found to occur on the fish all year in climate zone 3a ii.

Other species have a more limited annual occurrence, and must be assumed to survive during the months they do not occur on the fish by means of eggs which do not develop until optimum conditions return the following year. In some instances the species may survive the period of inclement conditions by very low levels of incidence on the fish. For each species the period of observed occurrence is given: *Ancyrocephalus percae*, January to March (Rautskis, 1970a), *Dactylogyrus alatus*, spring and autumn (Izyumova, 1964), *D. auriculatus*, spring and autumn (Izyumova, 1964), *D. cornu*, summer (Izyumova, 1964), *D. falcatus*, spring and summer (Izyumova, 1964), *D. fallax*, spring and summer (Izyumova, 1964), *D. minor*, autumn (Izyumova, 1960), *D. nanus*, spring and summer (Izyumova, 1964), *D. nasalis*, autumn (Strelkov *et al.*, 1969), *D. nobilis*, spring and autumn (Bauer *et al.*, 1969), *D. similis*, winter, spring

(Izyumova, 1960), *D. wunderi*, spring and autumn (Izyumova, 1964), *D. zandti*, spring and autumn (Bogdanova, 1958), *Gyrodactylus bychowskyi*, summer (Banina and Isakov, 1972).

It is of interest to note that Malmberg (1970) found *G. rarus* most of the year in Sweden but that Banina and Isakov (1972) found this species in the Neva delta reservoir only during July and September and concluded that it must be a warm water species. Malmberg kept it in the laboratory at a temperature between 4°C and 10°C all the year.

Concerning the biology of monogeneans, Bauer *et al.* (1969) reported that reproduction of *Dactylogyrus aristichthys* occurred at water temperatures from 14°C–15°C to 28°C–30°C. In the Moscow area they reproduced from mid-May until late August. A temperature of 22°C–25°C appeared to be the optimum. The duration of embryonic development was also determined by temperature, and freezing was shown to kill the larvae. Bauer *et al.* (1969) also summarised the information available concerning the reproduction of both *D. extensus* and *D. vastator*. The optimum temperature for *D. extensus* was lower than for any other species found on *Cyprinus carpio*. Eggs deposited at higher temperatures died, and young worms and larvae occurred during the winter. Prost's (1963) observations, reported earlier (Section IIIC1), were essentially identical to those summarised by Bauer *et al.* In *D. vastator* the optimum temperatures in the central zone of the U.S.S.R. were 22°C–24°C. This compares with an optimum of 28°C found by Paperna (1963) in the Mediterranean climate of Israel (Section IIIB1). In the U.S.S.R. eggs overwinter to hatch in the spring, the low temperatures slowing development to about 100 days (Bauer, 1959a). Lyaiman (1948, 1951b) demonstrated that adult *D. vastator* were found on *Cyprinus carpio* all year. The infections of *D. vastator* varied in relation to both season of year and age of the host (Lyaiman, 1951a,b).

It should be noted that although the temperature optima for *D. extensus* in both Poland and the U.S.S.R. were 16°C–17°C, Paperna (1964a) found that the optimum in Israel was 24°C–28°C. It is clear that temperature optima can vary through the range of distribution of a species.

3. *East coast* (Climate zone 3a iii)

This zone includes the eastern coasts of the United States of America from Cape Hatteras to the Gulf of St. Lawrence and the northern regions of Japan. No seasonal studies on freshwater monogeneans are known to the author from these areas. *Diplozoon nipponicum* studies reported by Kamegai (1970) on *Cyprinus carpio* may have been carried out in part in this climate zone. These studies have been reported in Section IIIB2.

4. *Marine west coast* (Climate zone 3b)

This zone includes western and north western Europe, the west coasts of Canada and the United States as far south as Cape Medocino, and in the southern hemisphere the west coast regions of Chile south of Valdivia, the Falkland Islands, Tasmania, parts of Victoria, Australia and much of New Zealand. The majority of seasonal investigations of freshwater monogeneans in this climate zone have been carried out in western Europe.

The following species were found during all months of the year, although in many instances the incidence and intensity was minimum during the winter months and maximum during spring and summer: *Dactylogyrus amphibothrium* (Wootten, pers. comm.), *D. auriculatus* (Wierzbicka, 1974), *D. chranilowi* (Wierzbicka, 1974), *D. cornoides* (Wierzbicka, 1974), *D. cornu* (Wierzbicka, 1974), *D. falcatus* (Wierzbicka, 1974), *D. macracanthus* (Wilde, 1935, 1937), *D. zandti* (Wierzbicka, 1974), *Diplozoon gussevi* (Wierzbicka, 1974), *D. nagibinae* (Wierzbicka, 1974), *D. paradoxum* (Zeller, 1892; Bovet, 1967; Mishra, 1966; Davies, 1967; Halvorsen, 1972; Wierzbicka, 1974), *Discocotyle sagittata* (Paling, 1965), *Gyrodactylus alexanderi* (Lester, 1974), *G. rarus* (Chappell, 1969).

Four species had different patterns of occurrence at different localities: *Dactylogyrus crucifer*, present all year (Mishra, 1966), absent September to January inclusive (Rizvi, 1964; Davies, 1967); *D. sphyrna*, present all year, (Wierzbicka, 1974), absent January, February (Mishra, 1966); *D. wunderi*, present all year (Wierzbicka, 1974), present summer months (Mishra, 1966); *Tetraonchus monenteron*, present all months (Rizvi, 1964), absent some months (Chubb, 1961; Mishra, 1966; Davies, 1967). These different results from different authors may represent fish sampling problems (for instance, Chubb, 1961 *Esox lucius*, or Mishra, 1966 *Abramis brama*) or variations from year to year, such as were seen for *D. zandti* by Wierzbicka (1974). Chubb (1961) was able to examine only young *Esox lucius* during some months. He showed that *T. monenteron* was found mostly on older *E. lucius*, hence it was not seen, although almost certainly present, during those months when only young *E. lucius* were caught.

The following monogeneans were present only at some periods of the year: *Ancyrocephalus paradoxus*, autumn, January (Wierzbicki, 1970); *Dactylogyrus cordus*, January to April, June to September (Davies, 1967); *D. distinguendus*, January to October (Wierzbicka, 1974); *D. fallax*, spring, December (Wierzbicka, 1974); *D. prostae*, February to August (Davies, 1967); *D. similis*, March to August (Rizvi, 1964); *D. vistulae*, February to August (Davies, 1967); *Gyrodactylus elegans*, May to September (Wierzbicka, 1974); *G. laevis*, summer months (Wierzbicka, 1974); *Tetraonchus borealis*, March to August, October, December (Davies, 1967).

Eggs were observed *in utero*: *Dactylogyrus amphibothrium*, February to August (Woolten, pers. comm.); *D. crucifer*, every month of the year (Mishra, 1966); *D. macracanthus*, all year (Wilde, 1935, 1937); *D. vistulae*, February to August (Davies, 1967); *Diplozoon paradoxum*, spring to autumn (Zeller, 1872), March to July, September to October (Mishra, 1966), February to November (Davies, 1967) and April to November (Halvorsen, 1972). Bovet (1967) noted that eggs produced during the cold months contributed little to the maintenance of the species, as diporpa larvae were first found in July and August. *Discocotyle sagittata* had eggs *in utero* during all months in Windermere (Paling, 1965) and maximum occurrence of juvenile worms was found during July and August. Longevity in Windermere was three to four years, but at Crummock Water maximum juveniles were found in November to December and longevity was only one year (Paling, 1965). Campbell (1974) found juvenile *D. sagittata* in

all months, but their incidence was maximal in November and December (41 %). *Tetraonchus borealis* contained eggs from March to July (Davies, 1967).

*Gyrodactylus rarus* were embryonated during November to June, not in August and September (Chappell, 1969).

Experimental observations about the effect of water temperature on some of these species are available. Wilde (1935, 1937) observed that the embryonic development of the eggs of *Dactylogyrus macracanthus* was dependent on temperature. In summer the larvae hatched on the 5th or 6th day, in March to April in 10 to 13 days and in winter in 18 to 20 days. The complete life-cycle took 14 to 16 days at 20°C–23°C, and about 30 days at 13°C–14°C. *Diplozoon paradoxum* was inactive during the winter and the genitalia did not function, but a rise in water temperature, naturally in spring, or artificially in an aquarium, even during winter, resulted in egg formation (Zeller, 1872). Bykhovskii (1957) found similar results in the U.S.S.R. Bovet (1967) agreed with Zeller's observations, and noted that the development of the embryo almost ceased at 4°C. If eggs were maintained at 4°C for a period and thereafter warmed to stimulate hatching, after 1½ months 80 %–100 % hatched, after 2 months, 50 %, and after 2½ months only 2 % (Bovet, 1967). Bovet found that a temperature of 24°C–26°C will kill *D. paradoxum*, but the fish host will survive. Halvorsen (1972) observed a sharp increase in gravid *D. paradoxum* concurrent with an increase in water temperature from 4°C in April to 10°C in May to June.

It is of interest to note that Malmberg (1970) found *Gyrodactylus anguillae* in western Sweden on elvers of *Anguilla anguilla*, but not on adult eels. How the parasite survives from year to year has not been explained, as elvers are distinctly seasonal in occurrence.

5. *Semi-desert* (Climate zone 3c)

This climate zone includes the states of Nevada, Arizona, New Mexico, Colorado and the eastern fringes of the Rocky Mountains in North America, the southern regions of European and Asiatic U.S.S.R., including parts of Iran and Afghanistan, and in the southern hemisphere part of Argentina, a small region in South Africa and in Australia a belt of country running from Peron Peninsular in the west, south to the Great Australian Bight and then further east to include much of New South Wales. Seasonal studies of monogeneans have been carried out only in the U.S.S.R. by Komarova (1964) in the Dnepr Delta, by Kashkovski (1967) in the Iriklin Reservoir on the River Ural and by Musselius (1967) in the Krasnodar Territory.

The following species were found during all the months of the year which were investigated: *Ancyrocephalus paradoxus*, *Dactylogyrus cornu*, *D. crucifer*, *D. simplicimalleata*, *D. sphyrna*, *Diplozoon bliccae*, *D. markewitschi*, *D. paradoxum*, *Tetraonchus monenteron* (Komarova, 1964). In each species the highest incidence and intensity of infection were during the spring, or more usually, the summer months.

Four of the species given in the previous paragraph had an alternative pattern of seasonal occurrence on different host species or in a different locality: *D. cornu* occurred April and September, *D. crucifer*, February to November, and *D. sphyrna*, June and August, all on *Rutilus rutilus* in the Iriklin Reservoir

(Kashkovski, 1967). *Diplozoon paradoxum* on *Rutilus rutilus heckeli* was found only in June, in contrast to its occurrence throughout the year on *Abramis brama* in the Dnepr Delta (Komarova, 1964).

The remaining species studied in this climate zone had some degree of seasonal occurrence. They were: *Dactylogyrus auriculatus*, February, April to June (Komarova, 1964); *D. cabolleroi*, April to August (Kashkovski, 1967); *D. falcatus*, March to May, July and August (Komarova, 1964), May (Kashkovski, 1967); *D. fallax*, February, May and June (Kashkovski, 1967); *D. haplogonus*, March to May (Komarova, 1964); *D. hypophthalmichthys*, April to October (Bauer *et al.*, 1969); *D. nanus*, April to July (Kashkovski, 1967); *D. ramulosus*, February to May (Kashkovski, 1967); *D. similis*, March (Komarova 1964), April and May (Kashkovski, 1967); *D. wunderi*, March to October (Komarova, 1964); *D. zandti*, May (Komarova, 1964); *Diplozoon homoion*, January to May (Kashkovski, 1967); *Gyrodactylus parvicopula*, May (Kashkovski, 1967). Some of the species given above were found on auxiliary hosts, and had the principal host species been investigated a different pattern of occurrence might have been seen.

There appear to be a few detailed studies on the biology of monogeneans in this climate zone. Bauer *et al.* (1969) reported that *D. aristichthys* reproduced at temperatures from 14°C–15°C to 28°C–30°C. In the Krasnodar Territory eggs were found from late April to September. Oviposition was most intensive at a water temperature of 22°C–25°C. As with all other monogeneans investigated, embryonic development time was determined by temperature. With *D. aristichthys* freezing killed the larvae. Development from egg to maturity lasted 11 to 13 days at 17°C–23°C.

6. *Desert* (Climate zone 3d)

No seasonal studies on monogeneans from this climate zone are known to the author.

7. *Sub-polar* (Climate zone 3e)

This climate zone includes large areas of northern Asia and North America. The interesting study by Rumyantsev (1972) in the Kuĭto Lakes in Karelia, U.S.S.R., was within this climate zone. Rumyantsev showed that the lower the spring and early summer temperatures of the water, the greater the displacement of the infection peak for *Dactylogyrus crucifer*, *D. fallax*, *D. nanus* and *D. similis*. The cold spring of 1962, when water temperatures in Lake Srednego Kuĭto did not exceed 10°C until after mid-June, resulted in the first appearance of *D. crucifer*, *D. fallax* and *D. nanus* in July. *D. similis* was not found even in July. In 1963 water temperatures above 10°C occurred by mid-May, and Rumyantsev found the four species of *Dactylogyrus* during May.

Shul'man *et al.* (1974) give data on the seasonal occurrence of *Dactylogyrus crucifer*, *D. nanus* and *Tetraonchus monenteron* from other Karelian lakes. Incidence was maximum during the summer months and they were not found during the winter.

### D. POLAR (Climate zones 4a and 4b)

The polar climates are unsuitable habitats for monogeneans in freshwaters.

### E. MOUNTAIN (Climate zone 5)

Vast areas of mountain country occur in North and South America and Asia. No seasonal studies on monogeneans from these areas are known to the author.

### F. SPECIES STUDIED IN MORE THAN ONE CLIMATE ZONE

A list of species which have been studied in more than one climate zone is given in Table II. Most of these species had a similar pattern of occurrence in

TABLE II

*Species of monogeneans studied (S) for seasonal occurrence in more than one climate zone. (The species are in alphabetical order.)*

| Monogenean species | Climate zone | | | | | | |
|---|---|---|---|---|---|---|---|
| | 2 | | 3 | | | | |
| | a | b | a i | a ii | b | c | e |
| *Actinocleidus fergusoni* | S | S | | | | | |
| *Ancyrocephalus paradoxus* | | | | S | S | S | |
| *Dactylogyrus amphibothrium* | | | | S | S | | |
| *D. aristichthys* | | | | S | | S | |
| *D. auriculatus* | | | | S | S | S | |
| *D. chranilowi* | | | | S | S | | |
| *D. cornu* | | | | S | S | S | |
| *D. crucifer* | | | | S | S | S | S |
| *D. extensus* | S | | S | S | | | |
| *D. falcatus* | | | | S | S | S | |
| *D. fallax* | | | | S | S | S | S |
| *D. hypophthalmichthys* | | | | S | | S | |
| *D. lamellatus* | | | S | S | | | |
| *D. macracanthus* | | | | S | S | | |
| *D. nanus* | | | | S | | S | S |
| *D. similis* | | | | S | S | S | S |
| *D. simplicimalleata* | | | | S | | S | |
| *D. sphyrna* | | | | S | S | S | |
| *D. vastator* | S | | S | S | S | | |
| *D. wunderi* | | | | S | S | S | |
| *D. zandti* | | | | S | S | S | |
| *Diplozoon paradoxum* | | | | S | S | S | |
| *Discocotyle sagittata* | | | | S | S | | |
| *Gyrodactylus elegans* | | | S | | S | | |
| *G. laevis* | | | S | | S | | |
| *G. rarus* | | | | S | S | | |
| *Tetraonchus monenteron* | | | | S | S | S | S |
| *Urocleidus ferox* | S | S | | | | | |

each climate zone, except that the lower temperatures for a longer period of the year in the more northern climate zones curtailed the season of reproduction. Unfortunately data about reproduction in the natural habitat were not always easy to obtain. Bauer *et al.* (1969) noted that *Dactylogyrus aristichthys* reproduced in the Moscow region (climate zone 3a ii) from mid-May to late August, but in the warmer conditions of the Krasnodar Territory (climate zone 3c) from April to September.

Other variations in occurrence between climate zones can also be partly explained by temperature. Some species, for example *Dactylogyrus auriculatus*, *D. cornu* and *D. falcatus*, were found predominantly during spring, summer and autumn in climate zone 3a ii (Bogdanova, 1958; Izyumova, 1960, 1964) but all the year in the milder winters of climate zone 3b. A similar situation existed for *Tetraonchus monenteron*. It occurred on *Esox lucius* all year in climate zones 3b (Rizvi, 1964) and 3a ii (Izyumova, 1964; Rautskis, 1970b) but not during the cold months, November to February, in the colder climate zone 3e (Shul'man *et al.*, 1974). Care must be taken with such generalisations, however, as within climate zone 3b, although Rizvi found *E. lucius* infected during each month of the year, Davies (1967) working in the same zone did not find infections during December and January. Often these differences can be explained by the interaction of two parameters. *Tetraonchus monenteron* was found more to be more abundant on older *E. lucius* (Kašták, 1957; Chubb, 1961). Thus Chubb examined *Esox lucius* in June and August, and they were not infected by *T. monenteron*, not because of a seasonal temperature effect, but because they were small fish. Had large fish been examined, they would have been infected.

Chappell (1969), in climate zone 3b, found *Gyrodactylus rarus* all year. In the colder climate zone 3a ii Banina and Isakov (1972) found this species only during July and September, and they concluded quite logically that *G. rarus* might be a warmth-loving species. However, Malmberg (1970), also working in climate zone 3a ii, found *G. rarus* as a natural infection during most of the year.

It is clear that some species of monogeneans can adapt to a changed habitat. Paperna (1963, 1964a) has described how *Dactylogyrus extensus* and *D. vastator* have become adapted to the warmer conditions of Israel (climate zone 2a). Experimental studies have also shown that some species prefer warmer or cooler conditions. Bauer (1959a) noted that *D. extensus* reproduced more successfully at lower temperatures than *D. vastator*. A lower incidence of *D. extensus* was noted during the summer by Ivasik (1953) and young worms and larvae were found during the winter.

## IV. General Conclusions

### A. Incidence and intensity of occurrence

From the information given in Section II it is clear that a considerable volume of information is available concerning seasonal incidence and intensity of infection of freshwater fishes by monogeneans. The field studies show that there is great variation in patterns of occurrence even within the members of a

single genus. Some species show minimal changes of incidence and intensity of occurrence during the year, others have minimal changes of incidence but considerable changes of intensity, whilst yet others have marked changes of both, often with periods of absence from the host. It is impossible to generalise, so each species should be considered separately. The patterns of incidence and intensity of infection can be related in a more or less precise manner to the effect of abiotic factors, oxygen concentration, water temperature, or biotic factors, host species, behaviour, migrations, immunity, or to the interaction of a range of both abiotic and biotic factors.

### B. REPRODUCTION

Where the information concerning the duration of the season of reproduction was given by the original research workers it is included for the species in Section II. A considerable variation exists, from species which reproduce continuously throughout the year, to those that reproduce for a part of the year only. Temperature has a major role with respect to the reproductive patterns of the monogeneans.

### C. LONGEVITY AND MORTALITY

Longevity cannot be determined exactly in field observations of the smaller species of monogeneans, but has been estimated for some larger species. *Diclybothrium armatum* lived one year (Bauer, 1959b), *Diplozoon paradoxum* lived at least two years in Switzerland (Bovet, 1967) but only one year in Norway (Halvorsen, 1969), and *Discocotyle sagittata* lived one year in Crummock Water, but for at least three (but not more than four) years in nearby Lake Windermere (Paling, 1965). These larger species reproduce seasonally during the warm months, but *D. paradoxum* will reproduce during the winter if the water temperature is raised to the necessary level (Zeller, 1872).

The longevity of the smaller monogeneans must be determined by experiment. Those investigated have a life of days to months, which decreases with increasing temperature. For instance, Lester and Adams (1974b) found that the longest life-span of *Gyrodactylus alexanderi* on *Gasterosteus aculeatus* kept under a photoperiod of 12h of darkness followed by 12 h of light (350 $\pm$ 150 lx) was 71 days at 7°C and 28 days at 15°C.

In the natural environment the populations of small monogeneans must be in a state of dynamic change, leading to the considerable variations of incidence and intensity which can be observed from month to month. Where incidence and intensity are observed to be the same for long periods, there must be a state of dynamic equilibrium, where the effects of reproduction, growth and mortality interact to give the observed stability of population.

### D. MORPHOLOGY

Malmberg (1970) found two forms of *Gyrodactylus pungitii*, one in brackish water, the other in freshwater. During warm water conditions the two forms

were very similar and difficult to distinguish, although they were separable during cool conditions. Other morphological changes which could be related to seasonal changes may occur. Gussev and Kulemina (1971a,b) have studied morphological, behavioural and developmental changes of monogeneans from fish of different ages.

### E. PRINCIPAL AND AUXILIARY HOSTS

When a parasite occurs in more than one host, it is almost always possible to observe that in one of them it is most frequent, grows to the largest size, reaches maturity most rapidly, produces the greatest number of eggs and, in general, appears to be best adapted to the conditions it provides, suffering no ill-effects from the restraining influences of the host (Dogiel, 1964). This is the principal host. In other auxiliary hosts, the parasite, although able to occur and reproduce, is less well adapted to the host. Although many monogeneans have a high degree of host specificity, some do infect more than one host. Where principal and auxiliary hosts occur the seasonal distribution of a monogenean may be different on each. If an accidental host is infected, that is a host on which the parasite occurs very rarely and develops only with difficulty, a third pattern of seasonal distribution may be found. As an instance of this, the occurrence of *Dactylogyrus falcatus* on *Abramis brama* (principal host) in the Dnepr Delta from March to August (Komarova, 1964) differed from its occurrence on *Rutilus rutilus* (accidental host) in the Iriklin Reservoir during May only (Kashkovski, 1957).

### F. HOST MICROHABITATS

Izyumova (1958b) observed that the occurrence of *Dactylogyrus simplicimalleata* remained unchanged during winter on *Pelecus cultratus* but it disappeared from the gills of *Abramis brama*. The difference was explained by the availability of oxygen in the different microhabitats in which the two species of fishes overwintered.

### G. HOST MACROHABITATS

In two closely situated habitats, different patterns of incidence and intensity of infection of the same host species may occur. An example of this phenomenon is found in the occurrence of *Dactylogyrus crucifer* on *Rutilus rutilus* in three habitats in England. Mishra (1966) found *D. crucifer* each month of the year in the Shropshire Union Canal. At Rostherne Mere, Cheshire, 35 km from the canal, Rizvi (1964) found *D. crucifer* from February to August only. Davies (1967) at the River Lugg, Herefordshire, 135 km from the canal, also found *D. crucifer* from February to August only.

### H. OVERWINTERING

Some species, for instance the larger monogeneans *Diplozoon paradoxum* and *Discocotyle sagittata*, overwinter as adult worms on the fish. Frequently

the smaller species do the same, for instance *Dactylogyrus lamellatus*, *Gyrodactylus alexanderi*, and many others. Some species may overwinter as either adults or eggs, for instance *Dactylogyrus vastator*, whilst yet others are not found on the fish during the winter but the eggs retain their viability at the bottom of the water to hatch as water temperatures increase in the spring, for instance *Dactylogyrus nobilis*.

For many of the species reviewed in Section II the exact manner of overwintering has not been described if the parasite is not present on the fish. It is assumed that the eggs will overwinter in the oviparous species. Exactly how the viviparous gyrodactylid species which have not been found on fish during the winter survive has not been established.

## I. LONG-TERM POPULATION CHANGES

Long-term population changes have not been studied to any significant extent. Campbell (1974) recorded the incidence of *Discocotyle sagittata* on *Salmo trutta* over a six-year period, and during this time the incidence varied, according to the year, from 20 % to 80 %, and intensity from 0·25 to 2 worms per fish. Wierzbicka (1974) provided data collected over two years for the species she studied. For *Dactylogyrus chranilowi* on *Abramis brama* the pattern of occurrence was essentially similar during 1969 to 1971. Rumyantsev (1972) has clearly shown how annual differences in water temperatures can influence the populations of *Dactylogyrus* species. The lower the spring and early summer water temperature, the greater the displacement of the incidence peak into the summer months.

## J. ABIOTIC FACTORS

Bauer (1959a) discussed the factors which influenced the occurrence of fish parasites on their hosts. Amongst the abiotic factors he included the amount of light entering the water, the depth, hydrogen ion concentration (pH), oxygen content, salinity, and temperature of the water. Engelbrecht (1963) added the factor of standing or running water. Prost (1959a,b) investigated the effect of some of these factors on the monogeneans in the River Vistula, Poland, and concluded that water currents, suspended particles in the water, low oxygen content and a high chloride and sulphate content of the water were unfavourable to the monogeneans. Changes in water temperature were also considered. However, although these factors are all relevant to the occurrence of a monogenean species in a habitat, not all of the abiotic factors have been demonstrated to have an effect on seasonal changes or patterns of occurrence.

Oxygen has been shown to influence the behaviour of *Dactylogyrus extensus* (Izyumova, 1958a). The occurrence of *Dactylogyrus simplicimalleata* on the gills of *Pelecus cultratus* during the winter but its absence on the gills of *Abramis brama* during the same period at the Rybinsk Reservoir, U.S.S.R., was attributed to the different oxygen levels available to these two species of fish in the dissimilar conditions in which they overwintered (Izyumova, 1958b).

Oxygen level does not appear to be a factor of major relevance to seasonal changes in occurrence of monogeneans.

Water temperature has been shown to be a major factor in monogenean biology. Experimental studies and observations have demonstrated the great significance of temperature at all stages of the life of the monogenean, determining the rate of oviposition, the speed of development of the embryo, the length of life of the oncomiracidium, the speed of maturation of the juveniles and the longevity of the adults of several species. Relevant references have been given in the preceding sections of this review. It is clear that temperature is the major abiotic factor in the determination of seasonal patterns of occurrence of the Monogenoidea.

From the experimental evidence available at this time it is possible to conclude that each species of monogenean has an optimum temperature at which all the life processes occur with maximum efficiency for the reproduction of the species. From the field observations on the seasonal changes of those monogeneans for which experimental information is also available it is evident that the period of maximum occurrence is concurrent with the time when optimum temperature conditions prevail. Such a situation has been shown to apply, for instance, for *Dactylogyrus extensus* and *Dactylogyrus vastator* (see Section II A1a) although, as will be noted later, biotic factors may modify this simple situation.

Temperature is relevant to all aspects of the biology of monogeneans. It has been shown to influence that status and distribution of dactylogyrids where they have been accidentally introduced by the uncontrolled transfer of fish (Bykhovskii and Izyumova, 1966), and epizootics of *Dactylogyrus* sp. and *Gyrodactylus* sp. have been related to season of the year, where temperature has been postulated as the limiting factor (Meyer, 1970).

## K. BIOTIC FACTORS

Biotic factors were discussed by Bauer (1959a) and Engelbrecht (1963). As monogeneans have direct life-cycles, no complications occur through changes in host feeding behaviour, although other biotic factors such as the density of the fish populations, reproductive behaviour, growth rates and migratory patterns could all modify seasonal occurrence. This has been shown with species of monogeneans with a more complex life-cycle, for example *Dactylogyrus iwanowi* and *Diclybothrium armatum* (see Section IIA1a and IIB1a respectively).

Cellular and humoral reactions of the host may also modify seasonal occurrence of monogeneans. The gill lesions, induced by several species of *Dactylogyrus* (*D. extensus*—Bauer & Nikol'skaya, 1954; Prost, 1963. *D. lamellatus*—Molnár, 1972. *D. macracanthus*—Wilde, 1937. *D. vastator*—Bauer, 1959a; Ivasik, 1953; Kulwieć, 1929; Paperna, 1964b; Spiczakow, 1930; Wunder, 1929), can cause the loss of worms. The sudden decline in the occurrence of *D. vastator* on *Cyprinus carpio* fry during late summer was explained by Bauer (1959a) in part by the loss of worms owing to the development of gill lesions, and in part by a change in behaviour of the fingerlings which restricted the

possibility of the *D. vastator* larvae making contact with the fish, which prevented further infection even though water temperature remained optimum. Vladimirov (1971) studied acquired immunity of fish to *D. vastator*. A superinfection of fish resulted in an immunity which lasted for more than two months. Immunisation of fish with *D. vastator*-precipitated antigen gave the treated fish an enhanced resistance to infection, owing to an activation of its defence mechanisms. The degree of immunity depended on the intensity of parasite infection, the temperature, and the species of fish. Lyaiman (1946, 1948) considered this immunity to be an age immunity, but with the knowledge of Vladimirov's study, it must be an acquired immunity.

In the investigation of fish immunology it was observed at an early stage that the antibody response was dependent upon the temperature of the environment. At low temperatures, 8°C–10°C, the response was slow or not detected, but at higher temperatures it occurred more rapidly. The effect of reduction in temperature was to prolong the latent period of antibody formation rather than to prevent antibody synthesis (Corbel, 1975). Such a variation in activity of antibody production, reflecting changes in water temperature, has been used to explain seasonal variations in incidence of some monogeneans. The high intensities of infection of *Lepomis macrochirus* by ancyrocephalans at a water temperature of about 9°C observed by Crane and Mizelle (1968) and Rawson and Rogers (1972b), of *Micropterus salmoides* by ancyrocephalans (Rawson and Rogers, 1972a) and of both species of fish by *Gyrodactylus macrochiri* (Rawson and Rogers, 1973) at similar temperatures, were explained by Rawson and Rogers (1972a,b) as being antibody production–temperature related phenomena.

Lester (1972), however, considered that a humoral immune response was not responsible for the loss of *Gyrodactylus alexanderi* from *Gasterosteus aculeatus* after the infection had been present for more than about two weeks. His reasons were that fish infected four weeks prior to the experiment lost flukes at a similar rate to previously unexposed fish, and the response was very rapid, as fluke numbers were controlled within two weeks, whereas Krantz *et al.* (1963) found that an immune response in *Salmo trutta* required three months to reach its maximum titre at 11°C, and intramuscular injections of whole-fluke antigen gave no protection to the fish. Lester considered the loss was more likely to be owing to the local tissue response to the presence of the parasite.

### L. SEASONAL STUDIES IN WORLD CLIMATE ZONES

An assessment of seasonal studies related to world climate zones has been given in Section III. Although not demonstrated for all species, temperature is likely to prove the most relevant single factor. Seasonal studies in tropical conditions are completely lacking, and information is urgently required. In the subtropics 17 species have been investigated, in the mid-latitude climates about 62 species. However, detailed studies with concurrent field observation and laboratory experimentation are few in number.

### M. AN HYPOTHESIS FOR SEASONAL OCCURRENCE

In order to stimulate research with a more positive aim, it is helpful to erect

an hypothesis which can be tested. If found wanting it can be replaced, and if correct it can be supplemented as further data becomes available.

Current evidence suggests that temperature of the water is the primary factor in an explanation of seasonal incidence, intensity and reproduction of the dactylogyrids and gyrodactylids. As noted earlier, temperature affects all stages of development. All other factors are normally secondary to temperature, although in certain conditions and seasons they may assume an importance equal to, but not eliminating, the effect of temperature. The effect of temperature determines the monogenean behaviour, reproductive capacity etc., but the other factors may restrict the basic monogenean bioactivity within limits which can be recognised. Figure 2 attempts to illustrate the hypothesis, for three hypothetical species A, B and C. Species A has a 25°C temperature optimum. At this temperature, longevity may be short, but oviposition maximal and survival of larvae and juveniles optimal. Accordingly, the population shown by the solid kite-diagram has maximum size. At temperatures above 30°C, and below 10°C, the conditions are unsuitable, and the population declines to

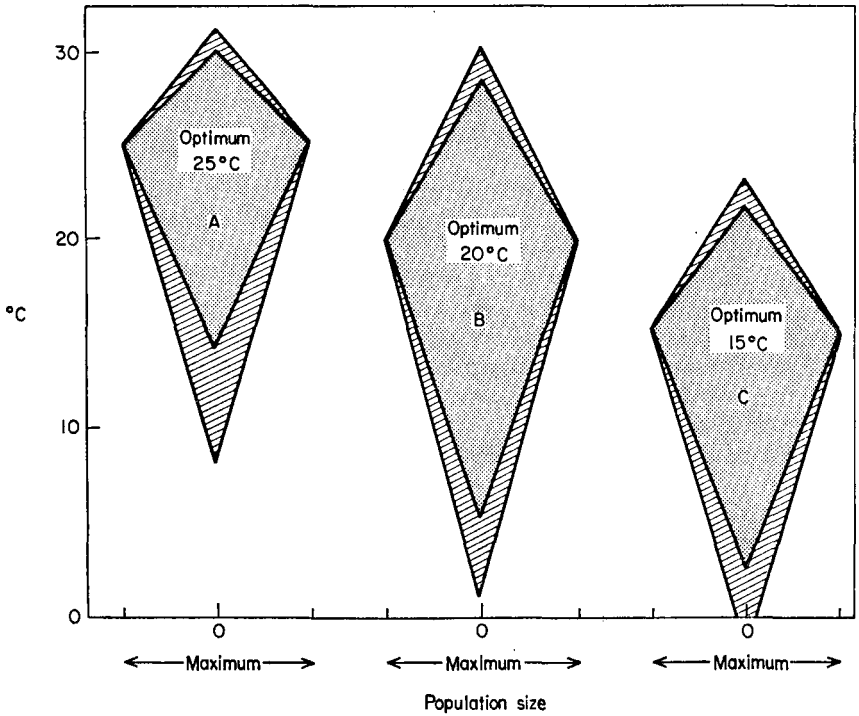



FIG. 2. Diagram to show population size in relation to temperature for three hypothetical freshwater monogenean species. Outer kites (striped) represent occurrence, inner (stippled) represent reproductive capacity. Population size at any season can be estimated by reference to water temperature.

a minimum. If the species lives in a habitat where water temperatures fall below 8°C, overwintering is by eggs. The same situation applies to species B, but in this instance, the temperature optimum is 20°C. Adult worms are present up to 28°C and down to 1°C. In the third species C the optimum temperature is lower at 15°C, and adult worms persist, in reduced numbers, during the winter, and are shown as being present down to 0°C. The hypothetical kite-diagrams correspond fairly closely to the actual patterns of occurrence of *Dactylogyrus aristichthys* (A), *D. vastator* (B) and *D. extensus* (C). The inner kites (stippled) show the reproductive capacity for the three hypothetical species. Reproduction is maximal at the optimum temperatures and ceases above and below certain temperatures. Again, the data shown in the figure correspond closely with the three actual species named in the previous paragraph.

The situation is usually more complex than that indicated above, and this may be shown by reference to *Gyrodactylus macrochiri* studied by Rawson and Rogers (1973). Figure 3 gives an indication of population size of *G. macrochiri*

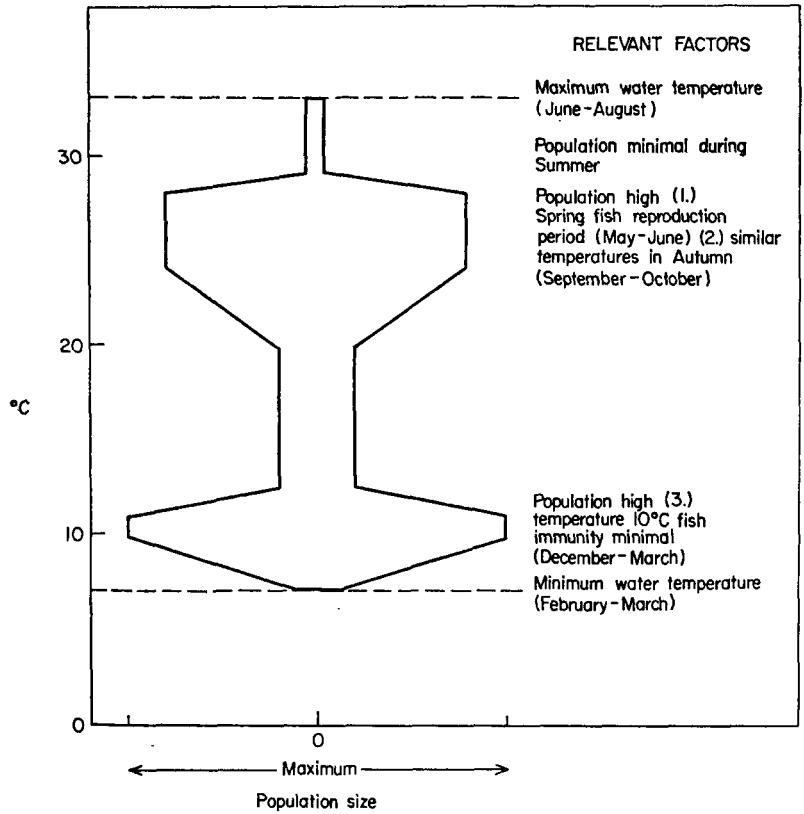



FIG. 3. Diagram to show population size of *Gyrodactylus macrochiri* on *Lepomis macrochirus* in relation to water temperature, based on the observations of Rawson and Rogers (1973). Reproduction was continuous throughout the year. (Months indicated in parentheses.)

on its host *Lepomis macrochirus*, following the trends of the data provided by Rawson and Rogers. At the right-hand side of the figure are listed the factors affecting population size indicated by Rawson and Rogers. As may be seen, maximum populations of *G. macrochiri* occur (1) during the spring reproductive period of the fish, at temperatures of 24°C–27°C, (2) during the autumn, at similar temperatures, and (3) during the winter when temperatures are below 10°C. The fish immunity may be minimal at this temperature (Rawson and Rogers, 1972a), although Lester (1972) discounted the effect of a host humoral response with *G. alexanderi.*

The larger monogeneans, such as *Diplozoon paradoxum* and *Discocotyle sagittata*, are also temperature controlled, but with a longevity of one or more years the population changes are more gradual. Reproduction may be restricted to warm conditions as in *D. paradoxum* or occur in cool conditions as in *D. sagittata.*

## N. EXPERIMENTAL STUDIES IN CONTROLLED CONDITIONS

The work of Lester and Adams (1974a,b) on *Gyrodactylus alexanderi* in a controlled temperature and photoperiod, to study the reproduction, longevity and mortality rates and produce a deterministic model which simulated the population changes on isolated fish, is relevant to the seasonal occurrence of monogeneans. Field observations, if made in a comprehensive and detailed manner, provide a great deal of information, but must be supplemented by carefully controlled experimental studies. A laboratory study, using convenient species of fish and their associated monogeneans, carried out over a range of temperature and other conditions, would provide considerable and detailed information concerning the factors which determine the seasonal occurrence of monogeneans. Such studies are likely to provide the most valuable data in the future, especially if related to concurrent field observations.

## ACKNOWLEDGEMENTS

I wish to thank Professor O. N. Bauer, Zoological Institute, Academy of Sciences, Leningrad, for his help in recommending relevant Russian literature. Grateful acknowledgement is made for the existence of *Helminthological Abstracts* and the *Index Catalogue of Medical and Veterinary Zoology*. Without abstracting sources such as these the task of preparing a reasonably comprehensive review would be immeasurably greater.

I am grateful to Geographical Projects, London, for permission to use the information about world climatic regions shown in Fig. 1.

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# The Host–Parasite Interface of Trematodes

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## I. Introduction

Trematodes, living as they do in a wide variety of habitats, may be in direct contact with host tissues, or are bathed in host fluids which might be secretory, circulatory or contain digestive enzymes. These enveloping structures and substances represent the source of food for the parasite and in some instances this host material is broken down extracorporeally by parasite enzymes prior to being taken in through the mouth. The method of feeding is essentially suctorial, in which food is sucked through the mouth into the gut and, because of the absence of an anus, the gut contents become egested through the same aperture.

The surface of trematodes is covered by a cytoplasmic tegument so that nutrients, in a micromolecular state, can be acquired also by this route, although this feature is probably more significant to nutrient uptake by the digeneans than to the other members of the Trematoda. The tegument, in addition, provides a route by which the parasite is able to secrete substances into the surrounding host environment, and may be involved also in other physiological activities such as excretion, ionic regulation and protection of the parasite from host enzymes. For a more detailed discussion of these more general features see Erasmus (1972).

Thus these two structures, the gut and tegument, represent regions of host–parasite contact and physiological exchange and can be regarded as representing the host–parasite interfaces of the trematodes. Within the last 3 years several exhaustive reviews have been published on the trematode tegument and it is intended to deal very briefly with this interface, simply emphasising new ideas which have been presented recently.

## II. The Trematode Gut

### A. The gut of larval trematodes

1. *The redial gut*

This digenean larval stage possesses a simple gut consisting of mouth, oral sucker, a short oesophagus and a simple, undivided caecum which might be short or extend through the entire length of the body. The active way in which redial stages ingest host tissues and other larval parasites is very clearly described by Lim and Heyneman (1972) in their discussion of intramolluscan intertrematode antagonism. The size of the caecum is relatively constant for a particular species, although the ratio of redial length to gut length may vary from 2:1 in very small rediae to 5:1 in the larger rediae (Rees, 1966). Thus, in the redial stage, two host–parasite interfaces occur, providing this stage with several surfaces for absorption and secretion.

The surface of the oral sucker and oesophagus of the redia of *Parorchis acanthus* (Rees, 1966) and *Fasciola hepatica* (Wilson, 1972) is lined by a continuation of the external tegument. In both of these species the oesophagus is surrounded by secretory gland cells which produce electron-dense, membrane-bound vesicles. The mode of discharge of the vesicle contents is a little uncertain, as Wilson (1972) was unable to detect any contact between the vesicle membranes and the luminal plasma membrane. The composition of the secretion is unknown, although Rees (1966) described the oesophageal glands in *Parorchis* as containing carbohydrate and protein.

There is an abrupt transition in the nature of the luminal surface from oesophagus to caecum. The caecum is lined by an epithelium (Krupa *et al.*, 1967a; Køie, 1971b) which is about 0·1–2·0 $\mu$m high in the case of *Neophasis lageniformis* (Køie, 1971b). The epithelium is cellular, long septate desmosomes linking adjacent cells. The cell cytoplasm is dense and contains a nucleus, granular endoplasmic reticulum (GER), free ribosomes, Golgi complexes (not observed by Krupa *et al.* (1967a) in *Cryptocotyle lingua*) and particles of alpha glycogen. The basal membrane of these cells forms tubular invaginations which project into the cell cytoplasm. Beneath the basement layer is a thin layer of muscles.

The most conspicuous feature of the caecal epithelium is the presence of projections up to 2 $\mu$m long, which arise from the plasma membrane. Both Krupa *et al.* and Køie describe these projections as folds, although Ginetsinskaya *et al.* (1966) interpreted similar structures in the caecum of the redia of *Petasiger neocommense* as microvilli. In *Neophasis* and *Cryptocotyle* these projections curve, sometimes branch and occasionally fuse again with the luminal plasma membrane. Krupa *et al.* (1967a) and Køie (1971b) imply that these folds enclose material present in the caecal lumen and that the "trapped" material is taken into the cell cytoplasm by means of pinocytosis. Although this hypothesis seems reasonable on a morphological basis, careful experimental analysis is needed for absolute proof.

There is no doubt that rediae actually ingest host tissues, and therefore utilisation of nutrients, derived from the breakdown of host tissues, could occur via two routes. The particulate remains of digested material could be acquired

and digested via pinocytosis associated with lysosomal activity. Soluble derivatives could be absorbed directly via the external plasma membrane, the complex folds providing a considerable increase in surface area for uptake.

Histochemical studies (Cheng, 1964; Probert, 1966; Køie, 1971b; Reader, 1971; Moore and Halton, 1975) provide some indication as to the physiological activities of this caecal epithelium. The relevant data are summarised in Table I. From this it can be seen that both acid and alkaline phosphatases are associated with the caecum, and in the redia of the *Cercaria imbricata*, leucine aminopeptidase activity was demonstrated by Reader (1971) although it was not present in *Fasciola hepatica* (Moore and Halton, 1975) or *Philophthalmus gralli* (Cheng and Lee, 1968). Probert (1966) commented on phosphatase activity of the host tissues in immediate contact with the oral sucker of *Echinoparyphium*. This activity could be derived from the regurgitated contents of the redial caecum and might possibly represent evidence for extracorporeal digestion of host tissues prior to ingestion by means of the oral sucker. Unfortunately, the limitations of histochemical techniques permit only limited assessments of the real potential of the redial caecum.

Evidence for the absorption of soluble nutrients has been reviewed by Erasmus (1972) and Hoskin and Cheng (1974). The latter authors demonstrated that the redia of *Himasthla quissetensis* is able to absorb and utilise glucose, although autoradiographic studies demonstrated the presence of labelled glucose only in the body wall, and not in the caecum.

Thus the real functions of the redial caecum have yet to be demonstrated. The absorption of soluble nutrients is a distinct possibility, but the intracellular digestion of host material by pinocytosis and intracellular digestion involving lysosomal sequences remains to be proven, although Moore and Halton (1975) suggest that the granules in the caecal gastrodermis of *Fasciola hepatica* associated with acid hydrolases are lysosomal in origin and probably function in intracellular digestion.

2. *The cercarial gut*

Basically this consists of a mouth surrounded by an oral sucker, a prepharynx, and a muscular pharynx followed by an oesophagus. There are two caeca which vary considerably in size in different species. The gross morphology of the cercarial gut and its variations have been discussed by Erasmus (1972). The cercarial gut usually contains fluid in which might be suspended globules and particles of uncertain identity and origin. Although elaborate in its morphology and ultrastructure, there is still very little evidence to suggest that the gut provides a functional component for the absorption of nutrients. The cercarial gut is derived *de novo* during the differentiation of the germ balls within sporocyst or redial stage. It does, however, in contrast to that of the redia, persist throughout the subsequent generations of mesocercaria, metacercaria and persists to provide the basis of the adult gut.

There exist relatively few descriptions of the ultrastructure of the cercarial gut. The first was that of the cercaria of *Schistosoma mansoni* by Ebrahimzadeh and Kraft (1969) which forms the basis of the account by Stirewalt (1974). Two other descriptions have been made by Køie, one of the cercaria of *Zoogonoides*

TABLE I

*Histochemical characteristics of the redial gut*

| Species | Acid phosphatase | | | Alkaline phosphatase | | | Leucine aminopeptidase | | | Esterase | | | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | W | L | OS | W | L | OS | W | L | OS | W | L | OS | |
| *Neophasis lageniformis* | ? | + | ○ | + | ○ | ○ | ○ | ○ | + | ○ | ○ | + | Køie (1971a) |
| *Echinoparyphium* sp. | ○ | + | ? | + | + | ? | n.t. | n.t. | n.t. | n.t. | n.t. | n.t. | Cheng (1964) |
| *Echinoparyphium recurvatum* | + | + | + | + | + | + | n.t. | n.t. | n.t. | ○ | + | + | Probert (1966) |
| *Cercaria imbricata* | + | ? | + | + | ? | + | + | ? | + | n.t. | n.t. | n.t. | Reader (1971) |
| *Fasciola hepatica* | + | + | ? | ○ | + | + | ○ | ○ | ○ | + | + | ? | Moore and Halton (1975) |

W. wa; L. lumen; OS, oral sucker.
+, positive reaction; ○, negative reaction; ?, no comment; n.t., not tested.

*viviparus* (Køie, 1971a) and the other of the intraredial cercaria of *Neophasis lageniformis* (Køie, 1973), on which the following is based. As described previously for the redial stage, the oral sucker, prepharynx, pharynx and oesophagus are lined by a syncytical tegument resembling, in its basic features, that covering the outer surface of the parasite. Similarly, there is an abrupt contrast between the termination of the tegument lining the anterior region of the gut and the start of the caecal gastrodermis.

The cercarial caecal epithelium resembles very closely the gastrodermis of the redia. The gastrodermis is cellular with lateral septate desmosomes and with the luminal surface thrown into long lamellae 25 nm thick and 1–3 $\mu$m long. The intracellular spaces may be distended and contain numerous small vesicles. A similar feature occurs in the cercaria of *Zoogonoides viviparus*. The basal plasma membrane forms tubular invaginations up to 0·5 $\mu$m deep.

The gastrodermal cells exhibit in *Neophasis* the characteristics of cells actively engaged in synthesis. The cells are rich in GER, Golgi complexes, mitochondria, lipid droplets and alpha glycogen particles.

Numerous membrane-bound vesicles are present and these are thought to be derived from the Golgi complexes. The vesicles are either small and electron dense or large and electron lucid. Køie (1973) suggests that the small vesicles fuse with the apical plasma membrane and discharge their contents into the caecal lumen. Secretory bodies have also been described from the caecal lumen of the cercaria of *Schistosoma mansoni* (Ebrahimzadeh and Kraft, 1969).

In *Neophasis* Køie has described membraneous whorls and rhomboidal crystals in the gut lumen and these are more abundant in the lumen of older, more mature stages. The whorls are not discharged from the cell cytoplasm but are formed by the rolling up of the external plasma membrane. The true significance of these structures is not clear but similar structures have been described in the adult digenean caecum and these will be discussed later. In *Zoogonoides* the caecum of cercariae still within the sporocyst, contains a dark amorphous material.

The free-swimming cercarial stage is in most cases very active and must expend a considerable quantity of energy in the process of host location, attachment, penetration or encystment in those species with a non-parasitic metacercarial stage. Most evidence (see Erasmus, 1972) suggests that the energy required is derived from endogenous sources, particularly glycogen, but it does seem anomalous that an apparently non-functional gut has the ultrastructural characteristics of a synthesising epithelium. The studies on *Zoogonoides* and *Neophasis* (Køie, 1971a, 1973) are of cercariae still within sporocysts and rediae, and it is possible that the ultrastructural features described may be related to the absorption and utilisation of the fluid surrounding the developing cercaria. Inevitably, one must turn to histochemistry for further information on the physiological state of the cercarial gut. Stirewalt and Walters (1964) demonstrated leucine aminopeptidase (LAP) activity in the caecum of the cercaria of *Schistosoma mansoni*, and Fripp (1967) the presence of esterase activity. Køie (1971a) records acid and alkaline phosphatase and aminopeptidase activity in the caecal cells of *Zoogonoides*. Moore and Halton (1975) demonstrated eserine-resistant esterase in the caecal cells

of the free-swimming cercariae of *Fasciola hepatica*. This activity was not present in cercariae still enclosed within the redia. There was no alkaline phosphatase activity in the cercarial caecum although the oral sucker was faintly positive. These authors were unable to demonstrate beta glucosidase, arylsulphatase, chymotrypsin-type protease or naphthylaminase in the cercarial stage. Cheng and Lee (1968) similarly were unable to demonstrate LAP activity in the caecum of the cercaria of *Philophthalmus gralli*.

In contrast, histochemical studies on larval stages by Janoff and Ford (1965), Probert (1966) and Krupa *et al.* (1967b) did not reveal any enzyme activity in the caeca of developing or mature cercarial stages.

3. *Post-cercarial development*

The cercarial stage in its subsequent development may encounter considerable change in its environment. These contrasts will involve exposure to varying osmotic stress, $O_2$ tensions and inevitably a change in diet. As far as available evidence suggests, the cercarial gut seems to be in a relatively quiescent state. In its entry into a contrasting environment, e.g. free-swimming cercaria into mammalian tissues, the cercarial gut must respond to the appropriate trigger or triggers so that it is able to deal immediately with a different type of food material. The nature of the stimulus/response mechanism which results in cellular multiplication and differentiation is as yet unidentified. At present information is available on gut development in two types of life-cycle represented by the genera *Schistosoma* and *Fasciola*.

(a) *Cercaria–schistosomule–adult*: Schistosoma mansoni. In this type of life-cycle, once the cercaria has penetrated the skin of the final host, there is continual development and differentiation culminating in the adult stage.

The gross changes in the development of the gut during this period have been described several times. The elongation of the caeca and posterior fusion of two caeca with the subsequent development of a single posterior caecum, represent clearly defined changes which have been used by several authors to distinguish growth stages obtained from both *in vivo* and *in vitro* culture. Cort (1921) originally described 18 stages of gut development for *S. japonica* and this was extended to 24 by Faust and Meleney (1924). The scheme was adapted for *S. mansoni* by Faust *et al.* (1934) and later by Yolles *et al.* (1949), with some slight modification. Clegg (1965) gave an account of schistosomule development in the mouse, showing that at 7 days post infection the caeca had begun to elongate and 4% had black pigment in the gut, indicating that they were feeding on red blood corpuscles. By the 15th day post infection, the caeca had fused posterior to the ventral sucker and most of the worms, now in the portal vessels of the liver, were feeding on red blood corpuscles. Bruce *et al.* (1971) gave an account of the ultrastructure of the gut of the hepatoportal stage, 27 days post infection. At this stage the caecum is well developed and both oesophagus and caecum contain partly digested host red blood corpuscles. There is still a need, however, for a detailed study of the gut of these young stages, and of the cellular and subcellular changes which occur associated with the onset of feeding and the digestion of complex molecules such as haemoglobin.

(b) *Metacercaria–adult*: Fasciola hepatica. The gross development of the gut during the growth of *F. hepatica* in the mouse has been described by Dawes (1962b). The ultrastructure of the gut of the newly excysted metacercaria has been described by Bennett and Threadgold (1973). At this stage the caecal lumen is irregular in shape and is enclosed by relatively few cells (3–4). The nucleated gastrodermis cells are joined at their apex by long sepate desmosomes. The luminal surface of each cell bears short, polymorphic structures described by Bennett and Threadgold as microvilli. The cytoplasm of these cells contains mitochondria, glycogen and extensive GER with narrow cisternae. No Golgi complexes were observed. The most distinctive feature of these cells is the presence of numerous large, dense secretory granules. The authors regard the gastrodermis at this stage as having the characteristics of a secretory epithelium and suggest that eccrine secretion of granules into the caecal lumen takes place so that the lumen becomes filled with a finely granular, homogeneous mass. The authors do not describe cycles of secretion and absorption and the epithelium is regarded as being secretory only at this stage.

The future development of this caecal epithelium is described by Bennett (1975) in a study of stages recovered immediately after penetration of the host intestinal wall, within the abdominal cavity of the mouse and finally from the liver. Those juveniles recovered 2 h post infection from the abdominal cavity had caecal cells resembling those of the newly excysted larva in most details. Stages recovered from the abdominal cavity up to 3 days post infection had caecal cells which changed in volume, gradually lost (via eccrine secretion) the large granules, and had a luminal plasma membrane which possessed microvilli at first and then developed lamellae. Golgi complexes were present and GER. The cell type was designated Type A by Bennett and is illustrated in Fig. 1A. After 3 days post infection juveniles were recovered from the hepatic parenchyma. The caecal cells gradually altered and assumed the characteristics of the cells from the adult caecum. The large granules (1·3 $\mu$m) were lost and the Golgi complex produced a smaller (0·5 $\mu$m) secretory granule. The apical plasma membrane was thrown into tall regular lamellae which projected into the caecal lumen. Bennett describes this cell type as Type B (Fig. 1B). Undifferentiated cells lying just above the basal lamina were regarded as being the precursors of the cell Type B.

Thus, in a period of several weeks, the adult gastrodermis is developed but the more obvious changes occur within the first week after infection. The loss of the large granules during migration of the juvenile through the host intestinal wall and across the abdominal cavity could be related to several functions: 1, to disrupt host cells and facilitate penetration; 2, to digest host cells after they have been burst by the action of oral and ventral suckers; 3, as a lubricant to facilitate movement. Bennett regards the first function as the most probable.

These morphological changes which occur during migration illustrate the change in gut structure which occurs in this period. Initially, the primary function of the gut appears to be secretion, and to this is added absorption once the parasite has reached the host liver. The juveniles contain stores of $\alpha$ glycogen which can be utilised until the absorptive function of the gastrodermis is properly established. The abdominal cavity and liver capsule differ con-

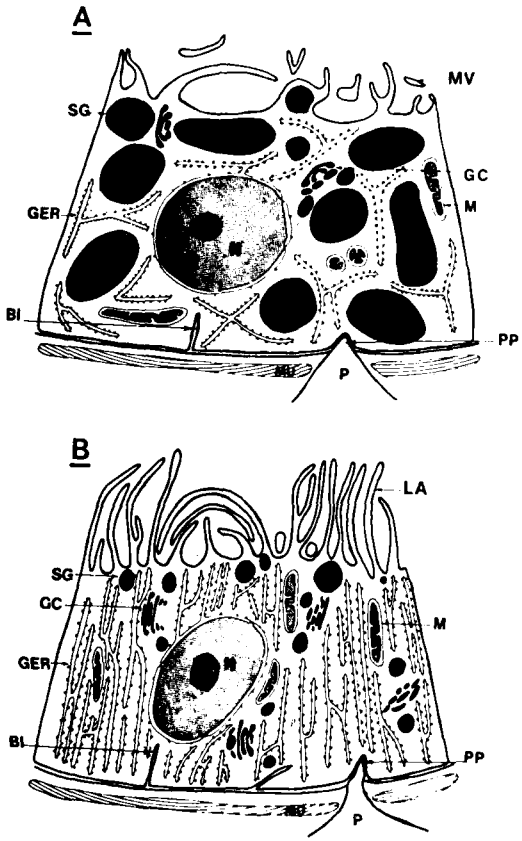



FIG. 1. The change in gastrodermal cell structure during the migration of *Fasciola hepatica* in the white mouse. A (above), Juveniles recovered from the abdominal cavity 3 days post infection; B (below), juveniles recovered from the liver capsule 3 days post infection. **BI**, basal invagination; **GC**, Golgi complex; **GER**, granular endoplasmic reticulum; **LA**, lamellae; **M**, mitochondrion; **MU**, muscle; **MV**, microvilli; **N**, nucleus; **P**, parenchymal cell; **PP**, parenchymal projection; **SG**, secretory granule. (Reproduced with permission from Bennett, 1975.)

siderably in their physiological and physical characteristics and the stimulus which triggers this change in gut function will not be easy to determine. As Bennett suggests, it could be a chemical substance specifically associated with the liver capsule, or a more general stimulus associated with the high nutrient content of the host liver. There are no histochemical data on the caecal epithelium characteristics of these early stages, but Thorpe (1968) has contrasted 3- and 5-week-old flukes recovered from the bile ducts with the adult stage. The only difference between the caecal cells of 3-week and adult individuals was the absence of glutamate dehydrogenase from the former. Alkaline phosphatase activity was absent from, and acid phosphatase present in, the caecal cells of all stages. Tests for non-specific esterase and leucine

aminopeptidase were negative. These results confirm the ultrastructural observations which suggest that by 3 weeks post infection the caecum has fully differentiated.

4. *Non-digenean larvae*

(a) *Aspidogastrea*—Multicotyle purvisi: In the larval stage of *M. purvisi*, Rohde (1971) found that the mouth, prepharynx and pharynx were lined with tegument similar in structure to that covering the external surface, but with the difference that the microfila were absent. The caecal cells have a luminal surface bearing numerous lamellae. The cytoplasm contains vacuoles, which either contain an electron-dense material or appear empty, and extensive GER. The cells are joined at their apices by demosomes.

## B. THE GUT IN ADULT TREMATODES

1. *The Digenea*

The earlier contributions to the interpretation of the digenean gastrodermis have been reviewed and discussed by Jennings (1968) and by Erasmus (1972).

From later studies a fairly general pattern emerges. The gastrodermis appears to be syncytial in most species with the quite definite exception of *Fasciola hepatica* in which the layer is truly cellular (Robinson and Threadgold, 1975). The situation in *Haematoloechus medioplexus* is a little uncertain in that both Dike (1967) and Davis *et al.* (1968) described septate desmosomes at the apex of adjacent "cells" but were not as definite regarding the presence of distinct lateral membranes separating the cells.

In all species the luminal plasma membrane is complex, providing a considerable increase in surface area. In most of the species described the evidence indicates that the surface elaborations take the form of lamellae which loop and fold and occasionally rejoin the luminal plasma membrane. In four genera, *Gorgodera* (Dike, 1967), *Gorgoderina* (Davis and Bogitsh, 1971b), *Megalodiscus* (Bogitsh, 1972) and *Diplodiscus* (Halton, 1966) it is apparent that the luminal surface is covered by microvilli. These are circular in cross-section and have supporting filaments. They vary in length from 20 $\mu$m (*Megalodiscus*) to 40 $\mu$m (*Gorgoderina*) and in diameter from 0·8–0·15 $\mu$m. Generally the plasma membrane of these structures is covered by a granular or filamentous glycocalyx. In all cases where a sufficiently detailed study has been undertaken, the basal membrane of the gastrodermis is heavily infolded forming tubular projections which penetrate deeply into the cell cytoplasm. There is also close contact between the base of the caecal cell and adjacent cells, and in some instances (*Fasciola*) finger-like extensions of parenchymal cells project into the cells (see Fig. 1). In the absence of a circulatory system this intimate intercellular contact might provide a route by which nutrients pass from the gastrodermis into the surrounding tissues.

The gastrodermal cytoplasm has an ultrastructure which reflects its metabolic activity. The cytoplasm is rich in GER, mitochondria and Golgi complexes. Various inclusions such as membraneous vesicles and electron-dense bodies occur and these seem to fall into two categories. The electron-dense bodies are

thought generally to have contents synthesised by the Golgi complex and are regarded as secretory in nature, whereas the membraneous complexes, residual bodies and membraneous vesicles seem to be associated with lysosomal activity and probably represent stages in cytosegresome formation. These suggestions are in the main based on morphological appearance although, in some species, histochemical and autoradiographic studies provide more conclusive evidence. This will be discussed later. This description corresponds in general terms to the static picture of the gastrodermis presented by most authors. However, in the case of *Fasciola hepatica*, more detailed studies have

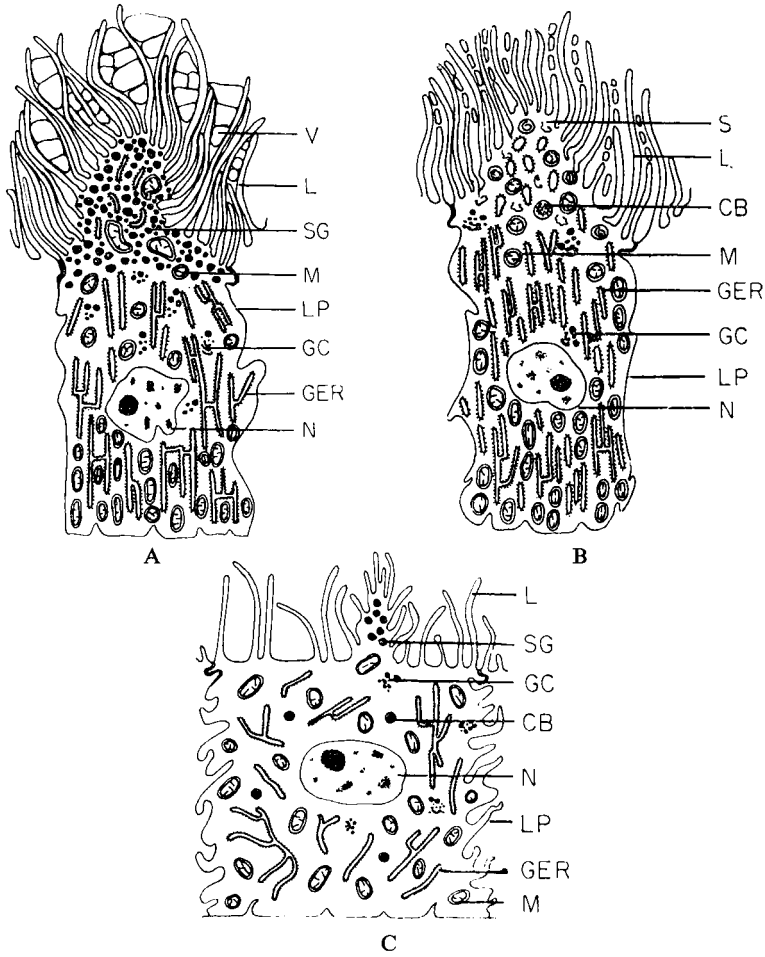



FIG. 2. Schematic diagrams of the cell types from the gastrodermis of adult *Fasciola hepatica*. A, Group I type cell; B, Group II type cell; C, Group III type cell. **CB**, cytoplasmic body; **GER**, granular endoplasmic reticulum; **GC**, Golgi complex; **L**, lamellae; **LP**, lateral plasma membrane; **M**, mitochondrion; **N**, nucleus; **S**, membraneous strip; **SG**, secretory granule; **V**, vacuole. (Reproduced with permission from Robinson and Threadgold, 1975.)

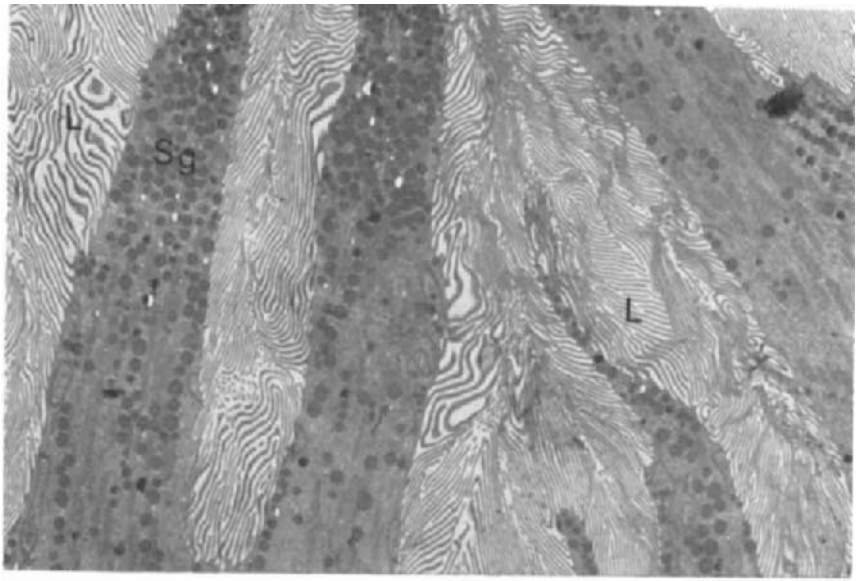
revealed a sequential change in the structure and the function of the cells of the gastrodermis. The most recent and detailed account is that of Robinson and Threadgold (1975).

Early studies at the light microscope level (Muller, 1923; Stephenson, 1947; Dawes, 1962a; Jennings, 1968) demonstrated that the cells of the gastrodermis exhibited a secretory cycle which was associated with the transformation of tall columnar cells (25–40 $\mu$m) into short cells (10–20 $\mu$m high). The gastrodermis must also have an absorptive function and the exact relationship between both these functions and the changes in morphology which occur has been difficult to determine. Robinson and Threadgold (1975) indicated that the variation in the morphology of the gastrodermal cells represents a continuing variation of a single cell. They established three major categories, Groups I, II and III (see Fig. 2). Group I contained the tall (25–40 $\mu$m) columnar cells having numerous lamellae at their luminal surface and containing a concentration of dense, secretory granules at their apex (see Fig. 3A, B). In this category of cells the Golgi complexes are abundant and active and are probably involved in the production of the secretory bodies. This group includes cells in various stages of activity described by the authors as ranging from early to late. In the late Group I cells, the granules are less numerous due to exocytosis of the granules of the luminal surface.

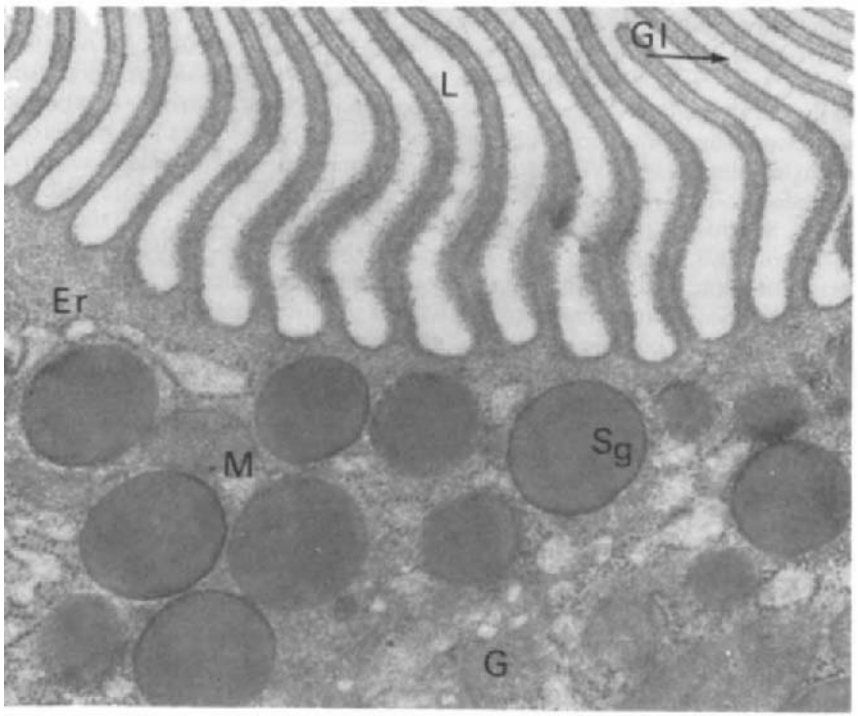
Group II cells are columnar but short (20–35 $\mu$m), with broad rounded apices, and have relatively few secretory bodies. The most characteristic feature of the cells is the presence of cytoplasmic bodies consisting of whorled membranes and whorled GER and perhaps mitochondria. The numerous apical lamellae, at certain phases of this cell group, show signs of disruption and breakdown. This group also contains a range of early intermediate and late stages, in which lamellae become reduced in size and number and the distribution of GER and mitochondria alters. At the early stage they are randomly distributed but, in the later stages, they become orientated in a basal–apical direction. Golgi complexes are relatively few and inactive and the spaces between lamellae contain exocytosed granules and membraneous whorls.

Group I cells are regarded by these authors as being secretory and are concerned primarily with the production of secretory granules and the release of their contents by an eccrine process. Group II cells are thought to be concerned with absorption. Late Group II cells exhibit a breakdown of the lamellae forming membraneous strips and whorls and it is suggested that these are endocytosed by the cell eventually giving rise to cytoplasmic bodies. By a lysosomal sequence this endocytosed material, with adsorbed host debris, will be digested and the soluble products will pass into the cell cytoplasm. This process does not exclude the possibility of micromolecular transport of nutrients via the apical plasma membrane. The situation is further complicated by the fact that some of the cytoplasmic bodies are associated with the sequestration and digestion of cell components which have reached the end of their viability.

The cells of Groups I and II comprise the gastrodermis lining the lateral caeca, and presumably secretion and absorption might occur principally in these regions. The main caeca are lined by a gastrodermis made up of cells of




A




B

FIG. 3A (above). Gastrodermis of adult *Fasciola hepatica*. The cell apices are filled with secretory granules (**Sg**) and a complex pattern of lamellae (**L**) protrude from the cell surface. × 3400. FIG. 3B (below). Detail of a secretory cell from the gastrodermis of *F. hepatica*. The cytoplasm contains secretory granules (**Sg**), Golgi complexes (**G**), mitochondria (**M**) and dilated endoplasmic reticulum (**Er**). The cell margin bears lamellae (**L**) which are covered with a filamentous glycocalyx (**Gl**). × 34 400.

Group III, which are cuboidal and 10–20 $\mu$m tall. The lamellae of these cells are short and both Golgi complexes and secretory granules are present. Cytoplasmic bodies which contain disrupted GER and mitochondria occur in the cytoplasm. The function of these cells is not clear. Robinson and Threadgold (1975) suggest that they may be concerned primarily with the movement of materials in the lumen of the gut. It seems likely that they can also be involved in secretion and absorption.

This sequence described for *Fasciola* is related to a distinctly cellular gastrodermis and it is interesting to consider whether or not similar differentiation occurs in the syncytial gastrodermis which appears to be more characteristic of the digenean gut (see Fig. 4A). Unfortunately, the absence of distinct lateral membranes does make clear demarcation into different phases more difficult to identify. Basal–apical orientation of components would not be maintained and lateral dispersion of secretory granules and lysosomal complexes will occur. Thus the syncytial gastrodermis consists of a mass of cytoplasm with increased surface area due to lamellae or microvilli and in which local physiological differentiation related to absorption or secretion must occur, but which cannot be related clearly to regional morphological categories. For some evidence supporting the idea of physiological differentiation of the caecal surface see Ernst (1975). In a study of acid phosphatase activity she described a direct relationship between the degree of activity demonstrated and the height of the gastrodermis and the degree of its folding. There was very little activity demonstrable in those regions where the gastrodermis was shallow and not folded.

(a) *The precaecal gut.* In contrast to the caecum, the anterior region of the gut comprising oral region, pharynx and oesophagus has received little attention. The most detailed accounts of the gut of *Schistosoma mansoni* are by Spence and Silk (1970), Morris and Threadgold (1968) and Dike (1971), and of *Schistosomatium douthitti* by Shannon and Bogitsh (1969b). The oral sucker is lined by tegument which in most details resembles that covering the general body surface. Within the pharynx the tegument becomes deeply fenestrated with long narrow folds of tegument. The folds are longer and narrower in the oesophagus and contain extensions of the basal plasma membrane in their centre (see Fig. 4B). The anterior oesophagus had a lining resembling tegument but the posterior oesophagus has additional distinctive features in that it contains characteristic secretory bodies within the tegumental folds. These are electron-dense rod shaped and rounded granules produced by the oesophageal glands. Morris and Threadgold (1968), Spence and Silk (1970), Dike (1971) and Ernst (1975) described the contents of the rounded granules as "crystalline" and also observed discharged, disintegrating granules in the channels between the long tegumentary folds. The lining of this anterior portion of the gut is tegumentary and is based on a plan similar to that of the external tegument, in being connected to cell bodies lying beneath the muscle layers. An additional dumb-bell shaped granule which is acid phosphatase positive has been described by Ernst (1975) in the posterior oesophagus.

In *Megalodiscus temperatus* the mouth, pharyngeal pouches and oesophagus

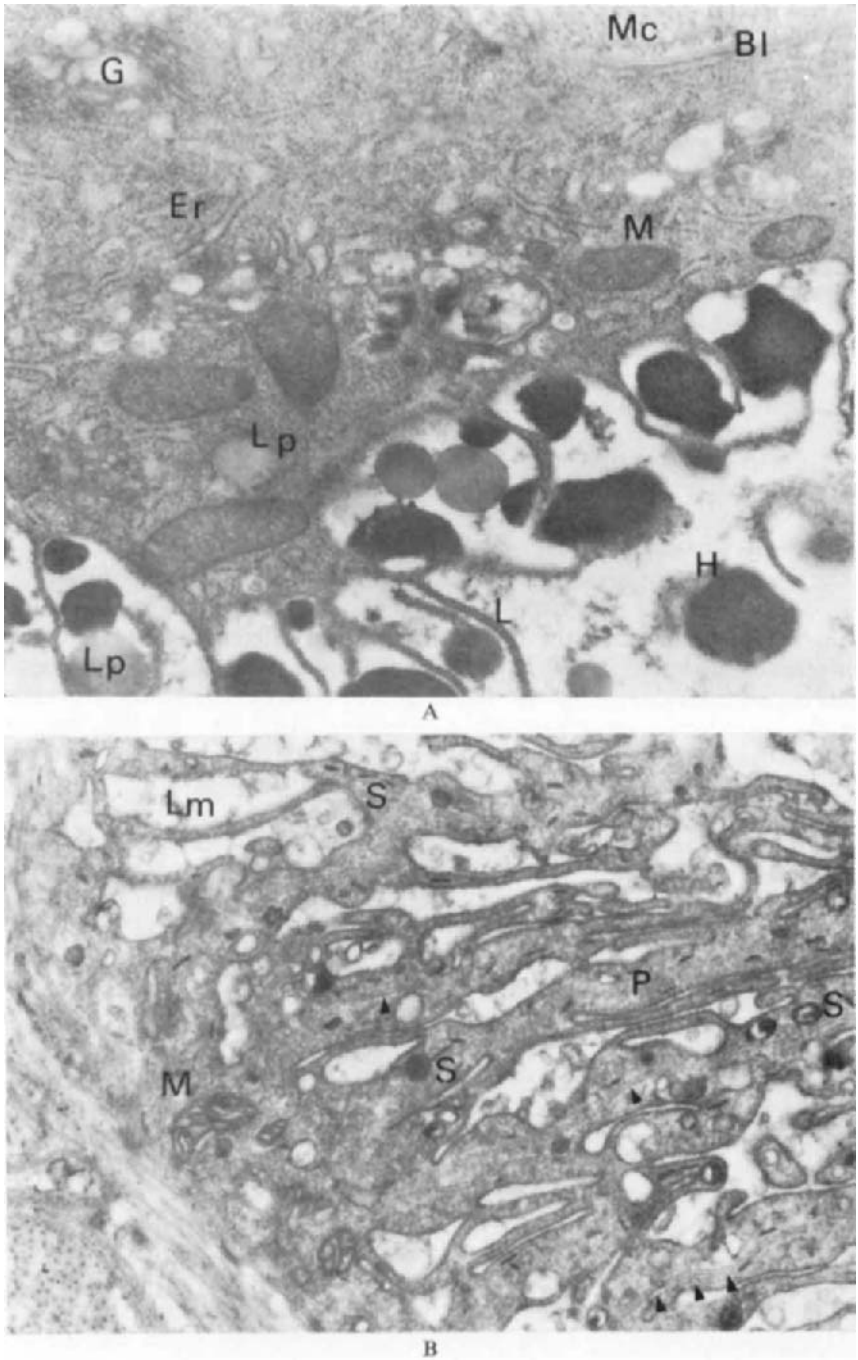



FIG. A (above). Gastrodermis of adult *Schistosoma mansoni*. The cell surface is elaborated to form lamellae (**L**) associated with which are lipid droplets (**Lp**) and haematin masses (**H**). The cytoplasm contains Golgi complexes (**G**), mitochondria (**M**), granular endoplasmic reticulum (**Er**) and lipid (**Lp**). The muscle coat (**Mc**) lies outside the basal lamina (**Bl**). × 22 100. FIG. 4B (below). Section through the oesophagus of *S. mansoni*. The tegument is extended into filiform processes (**P**) which contain a variety of secretory bodies (**S**), mitochondria (**M**) and invaginations of the basal lamina (arrows). The lumen (**Lm**) contains a variety of membranes and vesicles. × 20 800.

are covered by tegument. Oesophageal glands were not mentioned by Bogitsh (1972), who described numerous sensory structures in the region where the pharyngeal pouches arise. In *Schistosoma* and *Megalodiscus* the transition from tegumentary surface to gastrodermis is abruptly marked by a long junctional complex. Shannon and Bogitsh (1969b), however, do not describe a desmosome at the junction of tegument and gastrodermis.

Halton and Dermott (1967) have described large gland cells surrounding the oral sucker and pharynx of *Haplometra cylindracea* and *Opisthioglyphe ranae*. The cells are pear-shaped and have long ducts supported by microtubules, which pass through the tegument allowing the contents to be discharged to the exterior. The cells have the ultrastructural characteristics of secretory cells, i.e. abundant GER and numerous Golgi complexes, and because the cells eventually become packed and with electron-dense secretory granules, the process of secretion must be holocrine.

(b) *Histochemistry*. Histochemical and uptake studies provide further information regarding the functioning of the digenean gut.

Studies at the light microscope level reveal that alkaline phosphatase activity does not occur generally in the gastrodermis (Halton, 1967b and reviews by Jennings, 1968 and Erasmus, 1972), although Bogitsh (1972) obtained a positive reaction associated with the microvilli of *Megalodiscus*.

In contrast, acid phosphatase activity occurs consistently within the digenean gastrodermis and has been demonstrated in *Haematoloechus medioplexus*, *Haplometra cylindracea*, *Opisthioglyphe ranae*, *Diplodiscus subclavatus*, *Gorgoderina vitelliloba*, *Gorgodera cygnoides* and *Fasciola hepatica* (Halton, 1967b; Tarazona Villas, 1958; Saito, 1961; Threadgold, 1968; Fripp, 1966; Dike, 1969; Nimmo-Smith and Standen, 1963), in *Paragonimus westermani* (Yamao, 1952), *P. kellicotti* (Dike, 1969), *Clonorchis sinensis* (Ma, 1964), *Diplostomum spathaceum*, *Apatemon gracilis minor* and *Holostephanus lühei* (Öhman, 1965, 1966a,b) and *Megalodiscus temperatus* (Bogitsh, 1972).

Esterase activity has been recorded in the gastrodermis of *Fasciola hepatica* (Halton, 1967c), *Apatemon gracilis minor*, *Diplostomum spathaecum*, *Holostephanus lühei*, *Cyathocotyle bushiensis* (Erasmus and Öhman, 1963; Öhman, 1965, 1966a,b), *Alaria mustelae* and *A. marcianae* (Tieszen *et al.*, 1974), *Haematoloechus medioplexus* (Davis *et al.*, 1969), *Schistosoma mansoni* (Ernst, 1975) and *Haplometra cylindracea* (Halton, 1968).

Positive reactions for leucine aminopeptidase activity have been recorded in the gastrodermis of *Cyathocotyle bushiensis* (Erasmus and Öhman, 1963) and in the oesophageal glands of *Schistosoma rodhaini* (Fripp, 1967).

Naturally enough, all these authors have discussed the possible significance of these enzyme activities in relation to feeding, digestion and absorption in the caecum. Somewhat more significant information is provided by those studies in which the tests have been carried out at an ultrastructural level so that activity can be related more precisely to subcellular components. Some of these findings are summarised in Table II.

From this it can be seen that acid phosphatase is associated mainly with membrane and vesicles. It is understandable to link the acid phosphatase

TABLE II

*The ultrastructural localisation of enzymes in the digenean caecum*

| Enzyme | Lumen | Lumen folds | Microvilli | Golgi saccules | Golgi vesicles | Gran. endo. reticulum | Basal membranes | Vesicles, droplets |
|---|---|---|---|---|---|---|---|---|
| Alkaline phosphatase | ○(a) ○(g) | ○(g) | +(a) ○(g) | ○(g) | ○(a) ○(g) | ○(g) | +(a) ○(g) | ○(g) |
| Acid phosphatase | ○(a) ○(f) | +(d) ○(f) +(g) +(i) | +(a) ○(h) | +(a) +(b) +(d) ○(f) ○(h) | ○(a) +(b) +(d) ○(f) ○(h) | +(b) +(d) ○(f) ○(h) | +(b) ○(f) +(g) +(i) ○(h) | +(d) ○(f) +(i) +(h) |
| Thymine pyrophosphatase | ○(a) | | | +(a) | ○(a) | | ○(a) | |
| Nucleoside diphosphatase | ○(a) | | | +(a) +(b) | ○(a) +(b) | | +(a) | |
| Esterase | ○(e) ○(f) | ○(e) ○(f) | ○(e) ○(f) | ○(e) ○(f) | ○(e) ○(f) | ○(e) ○(f) | ○(e) ○(f) | +(e) ○(f) |
| Leucine aminopeptidase | ○(c) | ○(c) | ○(c) | ○(c) | ○(c) | ○(c) | ○(c) | ○(c) |
| Arylsulphatase | | | | | | | +(h) | +(h) |

(a) *Megalodiscus*—Bogitsh (1972); (b) *Megalodiscus*—Bogitsh (1973); (c) *Schistosoma rodhaini*—Fripp (1967); (d) *Haematoloechus, Paragonimus*—Dike (1969); (e) *Haematoloechus*—Davis *et al.* (1969); (f) *Haematoloechus*—Rothman (1968); (g) *Fasciola hepatica*—Threadgold (1968); (h) *Gorgoderina*—Davis and Bogitsh (1971a, b); (i) *Schistosoma mansoni*—Bogitsh and Shannon (1971).

activity of the luminal plasma membrane covering the microvilli or lamellae, with uptake. However, the real significance of phosphatase activity at these sites is not clear and Pappas and Read (1975), reviewing membrane transport in helminths, suggest that the close spatial proximity of surface hydrolases of *Hymenolepis* and *Moniliformis* to certain transport systems may give a kinetic advantage to these parasites for the absorption of hydrolysis products in providing local and very rapid hydrolysis. This advantage might enable the parasite to compete more effectively with the host for nutrients. There is no comparable information regarding transport systems at the caecal surface of digeneans, but it seems likely that this interpretation could have a more general application. An additional uncertainty is the origin of the hydrolases at the cell surface. In some instances, e.g. *Diplodiscus subclavatus*, Halton (1967a) concluded that the enzyme at the cell surface and in the lumen was derived from host cells. In the case of *Megalodiscus temperatus* (Bogitsh, 1972) it was thought that the acid hydrolases were endogenous to the parasite.

Robinson and Threadgold (1975) have suggested how these surface hydrolases may be involved in intracellular digestion in the case of *Fasciola hepatica*. The eccrine release of the electron-dense secretory bodies of Group III cells is associated with the breakdown and disruption of the surface lamellae. This membraneous material is endocytosed eventually giving rise to cytoplasmic bodies in the cell. These authors suggest that the cytoplasmic bodies become involved in a lysosomal–autophagic sequence in which the contents of the cytoplasmic bodies become broken down, thus releasing any nutritive material bound to the glycocalyx of the external plasma membrane. The soluble products of digestion would diffuse into the enclosing cytoplasm.

The function of hydrolases associated with the infolded basal membrane is also uncertain. Mettrick and Podesta (1974) have drawn attention to the possible significance of the infolding of the basal membrane of the tegument of *Hymenolepis* to the translocation of solutes in and out of the tegument. Once again it is possible that this system has a more general application, as there is considerable morphological similarity between the basal infolding of the tegument and the gastrodermis.

In complete contrast is the relationship proposed by Bogitsh (1975) between these basal infoldings of the gastrodermis and autophagy (see Fig. 5). Starvation of flukes produces a considerable increase in membraneous autophagic vacuoles exhibiting hydrolytic activity (Bogitsh, 1973—*Megalodiscus temperatus*; Davis *et al.*, 1969—*Haematoloechus medioplexus*; Bogitsh, 1975—*Schistosoma mansoni*). Starvation was also accompanied by changes in the hydrolase activity of the basal infoldings. Bogitsh (1975) has proposed, in *Schistosoma mansoni*, a complex scheme linking basal infoldings, GER, Golgi complexes and cytosegresomes in autophagic processes. Hydrolases are first synthesised in the GER and transported in vesicles to the Golgi complexes where they become incorporated eventually in primary lysosomes. These fuse with the outer membrane of the cytosegresome. The cytosegresomes are formed by the sequestration of a portion of cell cytoplasm by the infolding of the basal plasma membrane. The cytosegresome is thus enclosed by a double membrane and at this stage does not exhibit hydrolytic activity. This activity

appears later, after fusion with the primary lysosomes which discharge their contents into the intermembraneous space. The inner membrane breaks down and the hydrolases reach the enclosed contents. The undigested membraneous remains are discharged through the apical plasma membrane into the caecal lumen. In *Megalodiscus*, Bogitsh (1973) suggested that the infoldings of the basal plasma membrane which enclose cytoplasm and organelles represent a special type of flattened, smooth endoplasmic reticulum. In this instance, acid hydrolases are already present in the cysternae of the GER. It does seem that autophagy, following the classical processes described by De Duve and Wattiaux (1966) and Ericsson (1969), is a common feature of the digenean gastrodermis, as most authors refer to membraneous vesicles containing mitochondria or endoplasmic reticulum. However, the relationship between these intracellular processes and the acquisition of nutrients by the gastrodermis is not always clear.

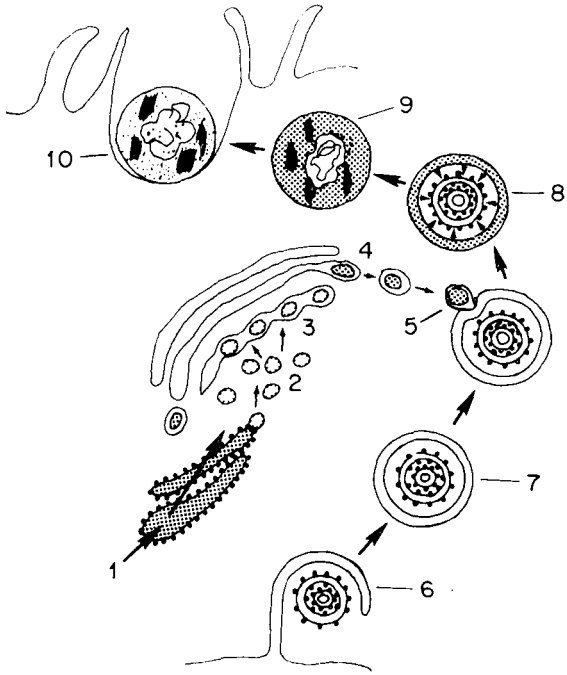



FIG. 5. A diagram showing the possible interrelationship between subcellular components in the process of gastrodermal autophagy in *Schistosoma mansoni*. The granular endoplasmic reticulum (**1**) synthesises hydrolases which are transferred in vesicles (**2**) to the Golgi complex (**3**). The hydrolases are concentrated into primary lysosomes (**4**). The lysosomes fuse with the outer membrane of a cytosegresome (**5**) which arises by the infolding of the basal plasma membrane (**6**). At this point the cytosegresome complex (**7**) does not possess hydrolytic activity. The primary lysosomes first release their contents into the intermembraneous space of the cytosegresome (**8**). The inner membrane eventually disappears and the enzymes mix with the enclosed cytoplasmic remnants forming a cytosome (**9**). Undigested material is exocytosed into the lumen as residual bodies (**10**). (Reproduced with permission from Bogitsh, 1975.)

The gastrodermis is characterised by inclusions which are described by different authors as secretory bodies and vesicles of different sizes. Two main functions have been associated with these structures. Because of the evidence which indicates that digestion in the digenean caecum is extracellular, it has been necessary to consider the possible source of the enzymes involved. As mentioned earlier, the enzymes might be exogenous, i.e. derived from the host or endogenous and arising within the parasite tissues. Unfortunately, the evidence available is rather ambiguous. The ultrastructural studies have not provided conclusive morphological evidence for an endogenous source of hydrolytic enzymes in most cases. The most convincing evidence for discharge of secretion is that of Halton and Dermott (1967) and Halton (1968) in their description of the acidophilic glands in the oral sucker of *Haplometra*. These cells were negative for esterase activity whereas the oral subtegumentary cells and tegument were strongly positive for acetylcholinesterase, but there was no indication as to how this material passed to the exterior. In the oesophagus of *Schistosoma mansoni* and *Schistosomatium douthitti* there is good morphological evidence which demonstrates the presence of secretory granules, derived from the oesophageal glands, within the lumen of the oesophagus (Morris and Threadgold, 1968; Shannon and Bogitsh, 1969b; Spence and Silk, 1970; Dike, 1971). Morris and Threadgold (1968) and Bogitsh and Shannon (1971) were unable to demonstrate acid phosphatase activity in these secretory granules, although the latter authors described acid phosphatase activity in vesicles associated with lysosomal activity.

Dike (1969) has described a membrane-bound dense granule and two other categories of inclusion in the gastrodermis of *Paragonimus* and *Haematoloechus*. She provided photographs which suggest that these granules in *Paragonimus* may pass through the external plasma membrane of the gastrodermis to the lumen of the caecum. She showed that acid phosphatase activity was present in all vesicles as well as in the Golgi vesicles. Davis *et al.* (1969) demonstrated the presence of non-specific esterase in the V2 vesicles of *Haematoloechus* and clearly showed the association of these vesicles with other cytoplasmic components, such as mitochondria, to form cytolysosomes. Residual bodies from this association were found near the luminal edge of the gastrodermis.

In *Fasciola hepatica* electron-dense secretory bodies, which are derived from the Golgi complexes, are clearly defined, and morphological and autoradiographic evidence for their discharge into the lumen is available (Robinson and Threadgold, 1975; Hanna, 1975). However, these bodies do not exhibit phosphatase activity (Threadgold, 1968) and the evidence for gelatinolytic activity presented by Thorsell and Bjorkman (1965) did not relate this activity directly to the secretory bodies and did not eliminate the activity possibly present in host debris already within the caecum.

In *Megalodiscus*, Bogitsh (1972) was able to demonstrate nucleoside diphosphatase and thiamine pyrophosphatase activity in the Golgi complexes and residual bodies though the apical vesicles present in the gastrodermis were negative. However, Bogitsh was able to show that macromolecular diglycols were present in the saccules of the Golgi complexes, Golgi vesicles and apical vesicles. Using autoradiographic techniques, Shannon and Bogitsh (1971)

showed that in *Megalodiscus* the Golgi complexes were the site for the packaging of carbohydrates which eventually became incorporated in the glycocalyx of the tegument. Bogitsh (1972) consequently suggested that the Golgi complexes and apical vesicles of the gastrodermis were involved in a similar function and contributed to the glycocalyx of the luminal plasma membrane. The inability to demonstrate the enzyme activity in these apical bodies may be due to the inefficiency of histochemical techniques rather than to a genuine absence of enzymes. In both *Haematoloechus* and *Gorgoderina* (Davis and Bogitsh, 1971a) arylsulphatase activity was demonstrated in vesicles involved in the formation of cytosegresomes.

Thus the histochemical evidence for autophagic processes within the gastrodermis is considerable, but support for the secretion of hydrolytic material into the lumen is very sparse although in some species, morphological and biochemical data are very suggestive.

(c) *Autoradiographic studies.* The gastrodermis also functions in absorption, and autoradiographic studies provide some information on this aspect of gastrodermal metabolism. In digeneans, absorption of soluble nutrients can occur through the tegument or gastrodermis and most studies have involved ligating the gut behind the oral sucker to differentiate the contribution made to uptake by these two routes. The consequences of ligation were undefined until the technique introduced by Halton and Arme (1971) enabled any areas of damage to be identified readily. A further study by Nollen and Nadakavukaren (1974), combining autoradiography with a scanning electronmicroscope (SEM) study of ligated adults of *Philophthalmus megalurus*, *Gorgoderina attenuata* and *Megalodiscus temperatus*, showed that ligation was an extremely unreliable technique which damaged the tegument in the region of the ligature. In some experiments they were also able to demonstrate, by autoradiography, leakage of tyrosine, leucine and thymidine in the region of the ligature. In view of these findings some reappraisal of earlier studies seems necessary. The role of the gastrodermis and tegument in amino acid absorption has been discussed by Pappas (1971), and the review of membrane transport systems by Pappas and Read (1975) emphasises our ignorance concerning activity at the luminal plasma membrane of the gastrodermis. These authors suggest that absorption of certain amino acids by *Fasciola hepatica* and *Schistosoma mansoni* is mainly transtegumentary. This conclusion is also supported by the study of methionine uptake by *S. mansoni* (Chappell, 1974) and of glycine and proline (Asch and Read, 1975). In this latter study the worms were suspended with the oral sucker excluded from the labelled culture medium.

It is apparent, however, that there is considerable variation between parasite species and the nature of the compound being absorbed. Pantelouris and Gresson (1960) showed that $Fe^{59}$ labelled ferric chloride accumulated in the gastrodermis of *Fasciola hepatica*, and others have demonstrated, in this fluke, the uptake of DL-methionine-$S^{35}$ (Pantelouris, 1964), DL-leucine-2-$C^{14}$, DL-phenylalanine-3-$C^{14}$ and DL-tryptophane-3-$C^{14}$ (Thorsell and Bjorkman, 1965) with subsequent localisation in the gastrodermis. In *Philophthalmus megalurus* Nollen (1968) suggested that glucose was absorbed through the

tegument, tyrosine and leucine mainly through the gastrodermis, and thymidine equally by both routes. Burton (1962) found little accumulation of radioglucose in the gastrodermis of *Haematoloechus medioplexus* and this was also confirmed by Parkening and Johnson (1969). Bhatti and Johnson (1971) were unable to demonstrate the uptake of labelled glucose, tyrosine and leucine by the gastrodermis of *Alaria marcianiae*, whereas Pappas (1971) demonstrated that in *Haematoloechus medioplexus* only the gastrodermis is active in the uptake of arginine. In *Gorgoderina attenuata* Nollen *et al.* (1973) found that as much tyrosine, thymidine and adenosine were taken up by ligated as by non-ligated flukes, indicating that gastrodermis and tegument function equally well in the uptake of these compounds. In contrast, Nollen *et al.* (1974) showed that, although thymidine and adenosine could be detected in the gastrodermis of *Megalodiscus*, maximum entry was through the tegument. In a study of iron absorption by *Haematoloechus medioplexus*, Shannon and Bogitsh (1969a) found that any iron acquired for metabolic purposes was ionic in form and entered the body via the tegument or gastrodermis. The haemoglobin from ingested host erythrocytes is broken down intracellularly and the iron-containing residues exocytosed as waste.

A completely different approach has been applied, by Hanna (1975), to the uptake and utilisation of amino acids by *F. hepatica*. These experiments involved the incubation of tissue slices of *F. hepatica* in culture media containing labelled amino acids. The four amino acids tested (tyrosine, methionine, leucine and phenylalanine) were found to become incorporated by the gastrodermal cells and the exact distribution of the label within the cell depended on the duration of the chase period. Within 0–10 min most of the label was found over the GER, especially in the region of the lateral and basal plasma membranes. After 20 min the label was most abundant at or near the Golgi complexes, and after a chase period of 30–45 min, labelling was predominantly in the apical region of the cells and associated with the secretory bodies, with the discharged secretory material at the luminal surface and within or between the lamellae themselves. This sequence suggests that the GER and Golgi complexes in the gastrodermal cells carry out a process of glycoprotein or protein synthesis via a sequence resembling that which occurs in mammalian cells. This investigation has also demonstrated clearly the release of the contents of the secretory bodies through the apical plasma membrane into the lumen of the gut.

The uptake, through the gastrodermis, of compounds with a high molecular weight has been studied by Thorsell and Bjorkman (1965), Rothman (1968) and Dike (1969), using ferritin and thorium dioxide markers. Thorsell and Bjorkman were unable to demonstrate uptake in the case of *F. hepatica*, Rothman found ferritin and thorium dioxide scattered sparingly in the gut of *Haematoloechus medioplexus*, and Dike (1969) described in this fluke the presence of ferritin associated with the luminal membrane and contents of the homogeneous cytoplasmic inclusions, and suggested that uptake was by phagocytosis and membrane-bound vesicles rather than by transmembranosis. Ernst (1975) was unable to demonstrate *in vitro* the uptake of horse-radish peroxidase, ferritin, thorotrast or latex beads by the oesophagus or caecum of male *S. mansoni*.

As well as the granules, secretory bodies and membraneous vesicles associated with the gastrodermis, lipid droplets occur fairly regularly and have received little comment other than by Morris (1968), who associated lipid droplets with the intracellular digestion of haemoglobin in *Schistosoma mansoni*. An increase in lipid in the vitelline cells of *S. mansoni*, associated with the presence of Astiban (Erasmus, 1975), also occurs in the gastrodermis.

Shannon and Bogitsh (1969b), in their description of the gut of *Schistosomatium douthitti*, included photographs of crystalline structures in the caecal lumen. Similar crystals have been observed in the gastrodermis of *S. mansoni* (Dike, 1971) and in both the gastrodermis and lumen of *Apatemon gracilis minor* (Blake, 1974). The crystals exhibit a lattice with regular spacing and, as the observations of *Apatemon* indicate, must be formed first in the gastrodermal cytoplasm and subsequently discharged into the lumen (see Fig. 6). The chemical nature of this proteinaceous material and its metabolic significance is far from clear. There is, of course, the possibility that it may represent the presence of viral infections, but discharge into the lumen of the caecum suggests a truly endogenous origin.

An isolated reference to the possibility of a sensory receptor system in the non-tegumental gastrodermis is made by Bogitsh (1975), who described a striated rootlet in the gastrodermis of *S. mansoni*. He postulates that this structure might be a component of a sensory network within the caecum by which exogenously derived stimuli (e.g. pressure of food or change in pressure of luminal contents) might be received by the gastrodermis, subsequently affecting the activity of subcellular components associated with intracellular digestion or autophagy. Further investigation of this possibility could be most rewarding.

Other inclusions observed in the digenean gut are virus-like particles in the gastrodermis of *Paragonimus kellicotti* (Byram *et al.*, 1975). These were rod-like or tubular, approximately 3·5 $\mu$m long by 35 nm in diameter and occurred in both cytoplasm and nuclear matrix. The cells containing these inclusions showed changes also in the morphology of the nucleolus and endoplasmic reticulum. Nuclear inclusions in the gastrodermis of *Megalodiscus* have been described by Morris (1973), and Dike (1971) has shown a paracrystalline arrangement of virus-like particles in the tegument of the oesophagus of *S. mansoni*.

In regarding the digenean gut as a host–parasite interface, through which interchange of substances with the host must occur, it seems reasonable to assume that the physiological activities of the gastrodermis may, in some way, become involved in the immunological response generated by the host. Digeneans are necessarily untidy feeders in which intake of host material by the sucking action of the pharynx alternates, as yet in an undefined manner, with the egestion of a complex mixture which might contain undigested host cells, partly digested host cells, exocytosed membranes or crystalline material and enzymes secreted from the gastrodermal cells. It seems likely that some of this mixture will be potentially antigenic. Some confirmation of this is the demonstration by Nash (1974), using fluorescence methods, of antigenic materials in the gastrodermis of adult *S. mansoni*.

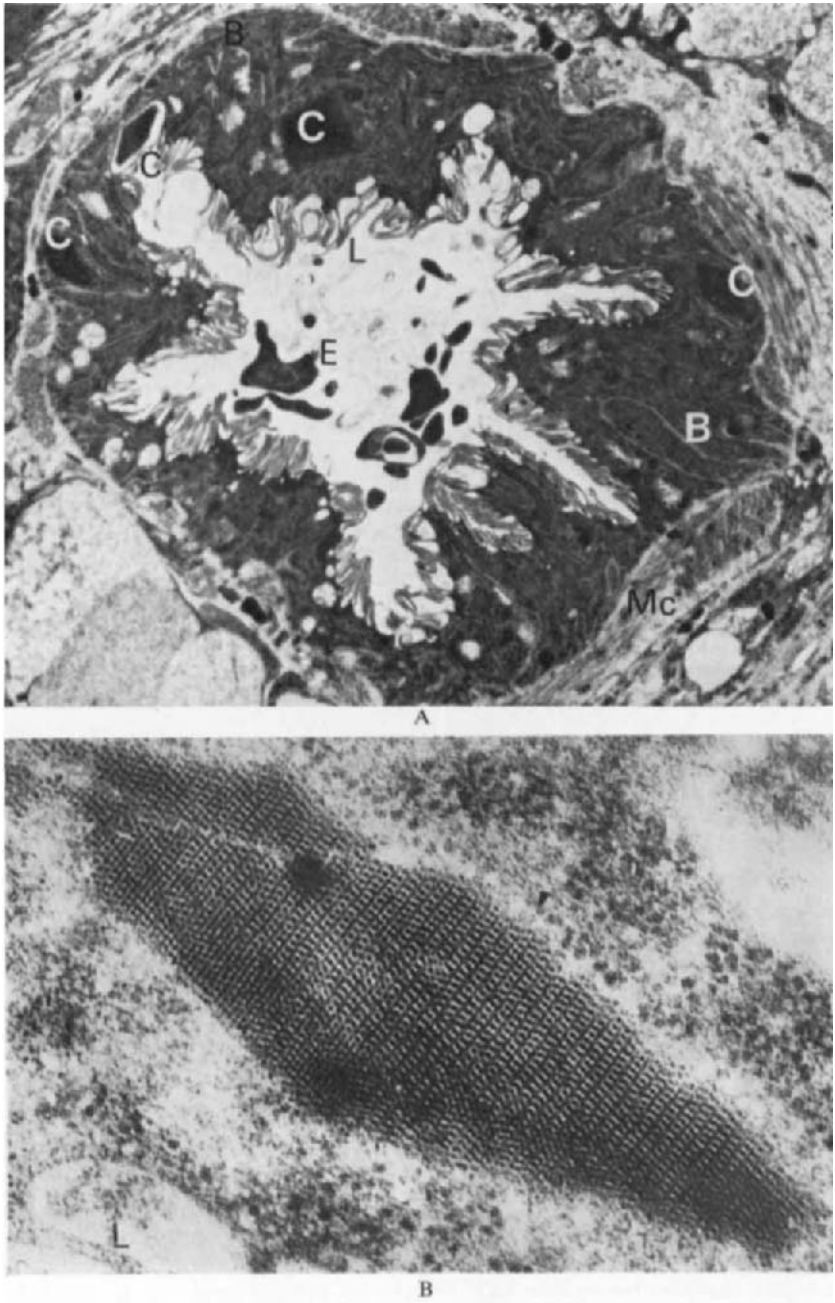



FIG. 6A (above). Section through the caecum of a 16-day-old *Apatemon gracilis minor*. The lumen contains avian erythrocytes (**E**) and cellular debris and the luminal surface of the gastrodermis bears lamellae (**L**). The cytoplasm contains crystalloid bodies (**C**) and the invaginations of the basal membrane (**B**) are conspicuous. The caecum is surrounded by a muscular coat (**Mc**). × 8000. FIG. 6B (below). Detail of a crystalloid body from the gastrodermis of *A. gracilis minor*. The regular spacing of the component units is very evident. **L**, lamella protruding into the caecal lumen. × 70 000.

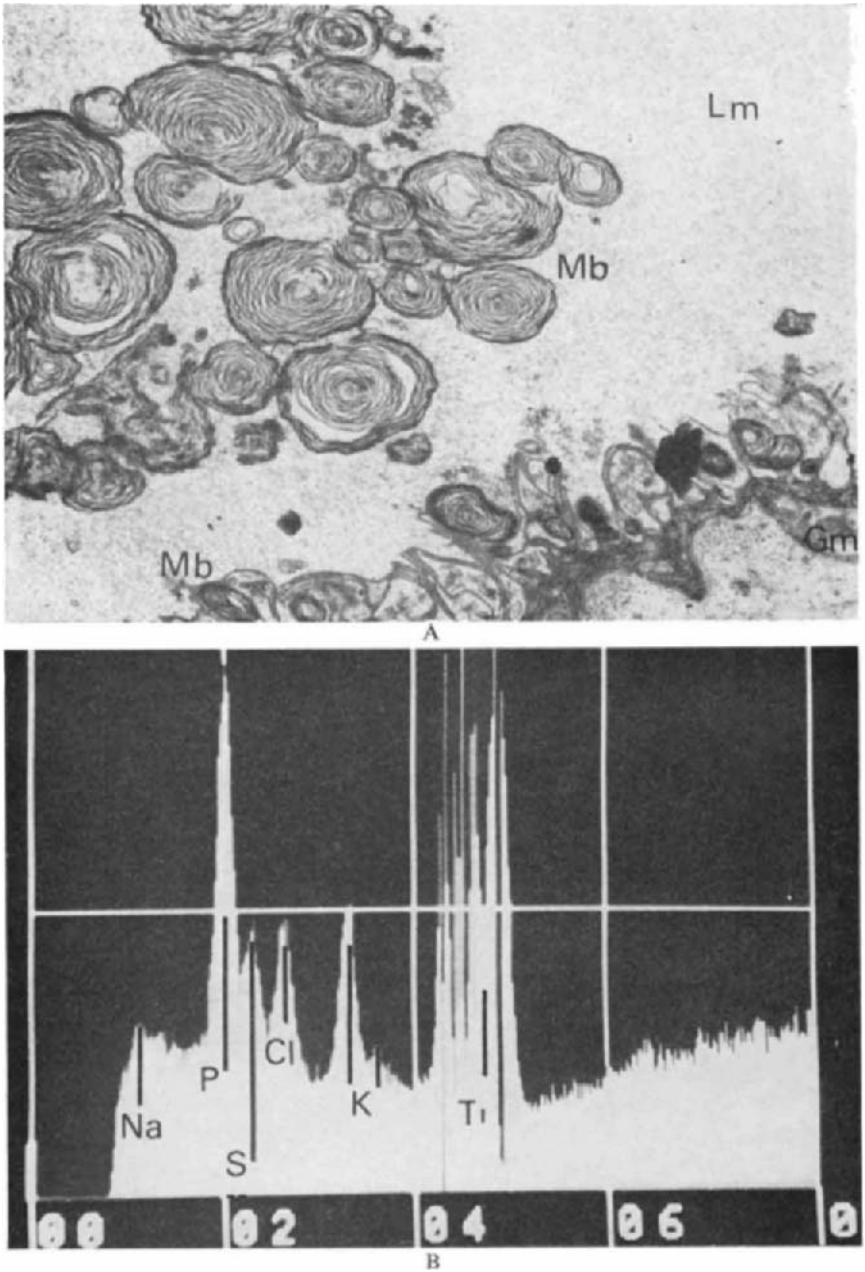



FIG. 7A (above). Section through the caecum of a 13-day-old *Apatemon gracilis minor*. The lumen (**Lm**) contains membraneous bodies (**Mb**) which appear to arise from the lamellae of the gastrodermis (**Gm**). × 9600. FIG. 7B (below). X-ray energy spectrum from a cryosection of spined tegument of female *S. mansoni*. The material was unfixed, quenched in liquid nitrogen, sectioned dry and analysed before staining. Spot size 3 $\mu$m, counting time 200 sec, full scale 1000 counts. The **Ti** peak is derived from the supporting grid bars. Elements present are sodium (Na), phosphorus (P), chlorine (Cl), sulphur (S) and potassium (K).

It is also probable that during the process of ingestion of host material, antibodies generated by the host will be taken into the caecum. The effect of antibodies on the tegument of *S. mansoni* has been reviewed by Hockley (1973). In a study of the immune response to *Apatemon gracilis minor*, Blake (1974) found that at the time (11–28 days post infection) when parasite rejection and host cell mobilisation were at a maximum, the caecal epithelium showed considerable change. The luminal plasma membrane of the gastrodermis became grossly elaborated to form masses of membrane whorls, which accumulated in the caecal lumen (see Fig. 7A). These membraneous whorls were also present in the gastrodermal cytoplasm. The changes at the surface of the gastrodermis resemble, to some degree, the membraneous bodies present in the outer plasma membrane of the tegument of specimens of *S. mansoni* exhibiting immunological damage.

(d) *Effect of drugs on the gastrodermis.* In addition to antibodies, the gastrodermis will come into contact with drugs which are present in the lumen of the digestive tract or circulatory system of the host. Bogitsh (1975) describes an increase in the incidence of autophagy in the gastrodermis of *S. mansoni* after exposure to hycanthone under *in vitro* conditions. This response was observed also under conditions of starvation but the process was accelerated by the presence of the drug. There was an increase in the number of Golgi complexes, secondary lysosomes and cytolysosomes which exhibited acid hydrolase activity. The cytoplasmic bodies present in the cytoplasm were membrane bound and contained whorls of GER and mitochondrial remnants.

In a study of the effects of Astiban on the vitelline cells of *S. mansoni* (Erasmus, 1975) it was also observed that within 3 h of a single intraperitoneal dose of the drug the gastrodermis of *S. mansoni* showed a response resembling that described by Bogitsh for hycanthone (see Fig. 8). In the Astiban material, however, it was noted that the cytosegresome remnants were discharged into the caecal lumen. Erasmus (1975) has shown that the cytosegresomes which appeared in the vitelline cells after drug treatment accumulate antimony, and it was suggested that this could represent a process of drug sequestration. Although analyses to demonstrate the presence of antimony in these gastrodermal cytosegresomes have not yet been undertaken, it seems possible that a process of drug sequestration and elimination might occur in the gastrodermis.

Bogitsh (1975) presents a hypothesis describing the origin of these cytosegresomes (see Fig. 5). The hydrolases, synthesised in the GER, are transported via vesicles that fuse in the vicinity of the Golgi complexes. Primary lysosomes containing these hydrolases are released from the Golgi complexes and fuse with the outer membrane of a cytosegresome. The cytosegresomes are formed by the sequestration of a portion of gastrodermal cytoplasm by an infolding of the basal plasma membrane. The undigested residues within the cytosegresome are finally ejected into the caecal lumen as residual bodies. It does seem possible, therefore, that as in the vitelline cell of *S. mansoni* (Erasmus, 1975), the process of sequestration of cellular components involving lysosomal activity characteristic of the metabolism of the normal gastrodermal

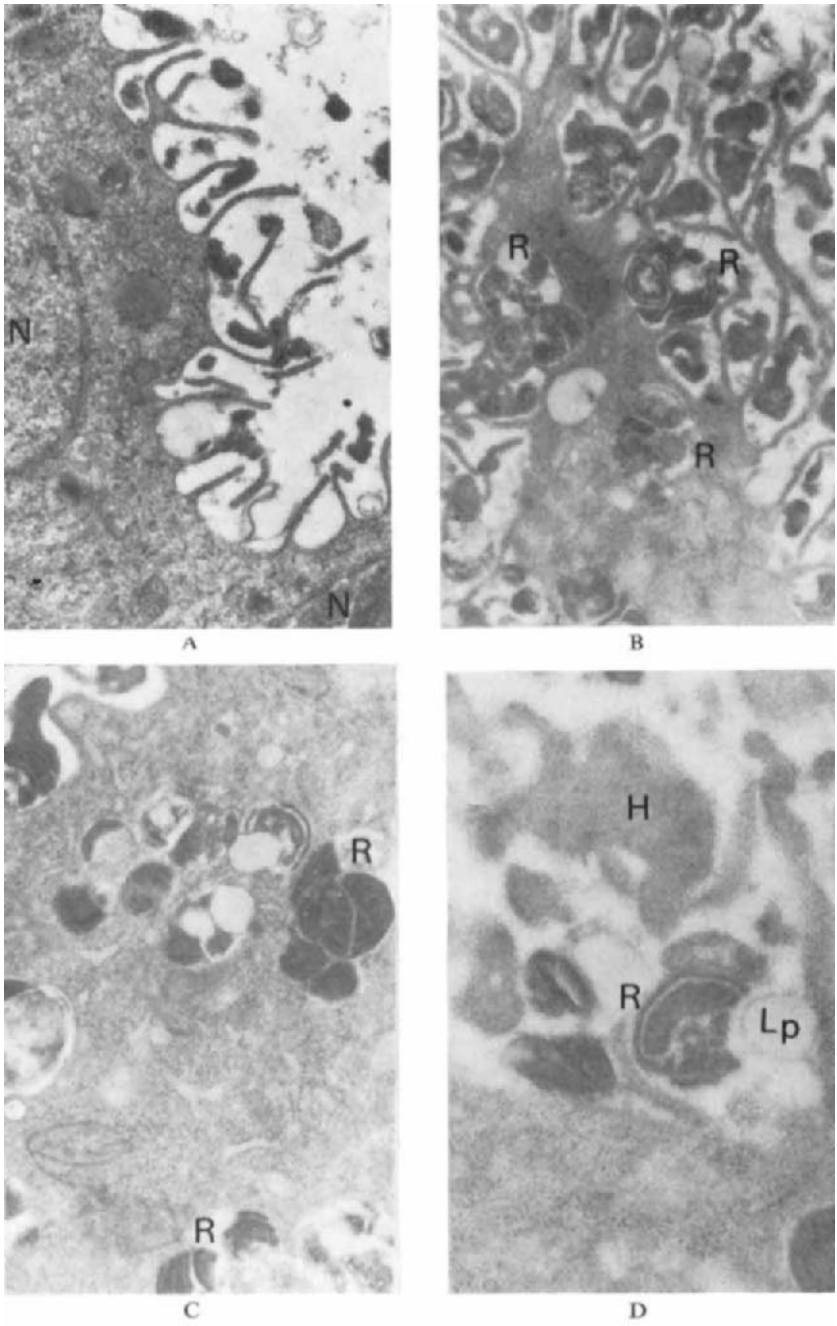



FIG. 8A. Gastrodermis of adult *S. mansoni* showing nuclei (**N**) and normal cytoplasmic inclusions. × 50000. FIG. 8B. Gastrodermis of a worm 3 h post injection of a single dose (40 mg/kg) of Astiban. Ribosomal complexes (**R**) are present within the cytoplasm and between the lamellae. × 13000. FIG. 8C. Details of ribosomal complexes (**R**) within gastrodermis. × 20 800. FIG. 8D. Ribosomal complexes (**R**) in lumen of caecum adjacent to lipid droplets (**Lp**) and haematin masses (**H**). × 44 400.

cytoplasm, may become enhanced under certain conditions and provide a route by which drugs are eliminated from the parasite tissues.

2. *The Aspidogastrea*

The ultrastructure of the alimentary tract of *Aspidogaster conchicola* has been described by Halton (1972) and a general review has been published by Rohde (1972). Trimble *et al.* (1971) have described the distribution at light microscope level of acid and alkaline phosphatase activity in the tissues of *A. conchicola*.

As far as can be judged from the detailed account by Halton (1792), the gut of *Aspidogaster* resembles in gross terms the ultrastructure of the digenean gut. The mouth, pharynx and oesophagus are lined by tegument which is similar to the tegument covering the general body surface. The junction between gastrodermis and oesophagus is marked by a long, tight intercellular junction.

The gastrodermis itself is cellular and consists of a single layer of columnar cells approximately 30–40 $\mu$m tall by 5–6 $\mu$m in diameter. The luminal surface of each gastrodermal cell possesses lamelloid folds and the basal plasma membrane is infolded into the cell cytoplasm. The epithelium rests on a basal lamina and has externally an inner circular and outer longitudinal series of muscles. Some of the gastrodermal cells contain electron-dense basophilic granules which are membrane-bound and up to 0·5 $\mu$m in diameter. The cells, which are rich in granules, also contain numerous Golgi complexes and extensive GER and thus exhibit the morphological characteristics of a protein secreting cell. Other cells are rich in lipid droplets, have their mitochondria basally oriented and have few small Golgi complexes. These cells gradually undergo cytoplasmic disintegration and in this process the GER produces large numbers of vesicles containing electron-lucid, fluid-like contents. These vesicles release their contents so that the apex of the cell contains a homogeneous granular material. The cells acquire a bulbous tip containing granular material and lipid and these cell apices become separated from the main body of the cell and are released into the caecal lumen. These cells contain crystalline inclusions similar to those described from the digenean gut, and also myelin figures which occur less often in the cells containing the electron-dense granules.

Thus the gastrodermis of *Aspidogaster* exhibits the ultrastructural features of an epithelium which is both synthetic and secretory and in which cyto-segresome formation occurs. The observations of Trimble *et al.* (1971) indicate that the gastrodermis is devoid of alkaline phosphatase activity but exhibits acid phosphatase activity in the pharynx tegument and also intracellularly in the gastrodermal cells. The observations of Trimble *et al.* (1972) on this same species reveal that the gastrodermis also contains a mixture of cholin-esterase and Type C esterase or cathepsin.

3. *The Monogenea*

In the digenean and aspidogastrean gut, all the evidence suggests that digestion is extracellular occurring in the caecal lumen. This is in complete

contrast to the situation in the Monogenea in which digestion is intracellular in its final stages—at least in those species which have been described. Halton and Jennings (1965), in a discussion on the nutrition of monogeneans, indicated that the Monopisthocotylea and the Polyopisthocotylea differ in their diet, the former being tissue and mucus feeders whilst the latter are sanguinivorous. This study was conducted at the light microscope level and on that basis the authors indicated that the gastrodermal structure also differed between these two major groups. The Monopisthocotylea had a continuous gastrodermis whereas the Polyopisthocotylea exhibited a discontinuous epithelium in which cells packed with haematin granules were separated by areas apparently devoid of cells. Recent ultrastructural studies by Rohde and by Halton and his co-workers have provided more precise and informative data on the gut of these two major monogenean groups.

In a study of *Diclidophora merlangi*, Halton and Morris (1975) showed that the anterior portion of the alimentary tract is lined by a linear sequence of morphologically distinct epithelia (see Fig. 9). The mouth and buccal cavity are lined by modified tegument which possesses uniciliate sense receptors and has, in the buccal region, a spiny appearance. This portion of the gut receives the openings of the buccal glands which produce a secretion consisting of acidophilic protein-rich droplets (Halton *et al.*, 1974).

The prepharynx is lined by an epithelium which is folded and varied in structure in different regions. This region also receives the ducts of gland

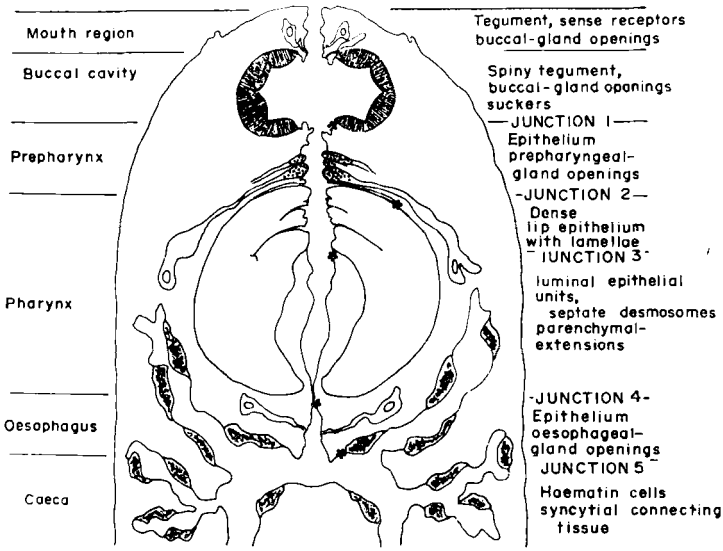
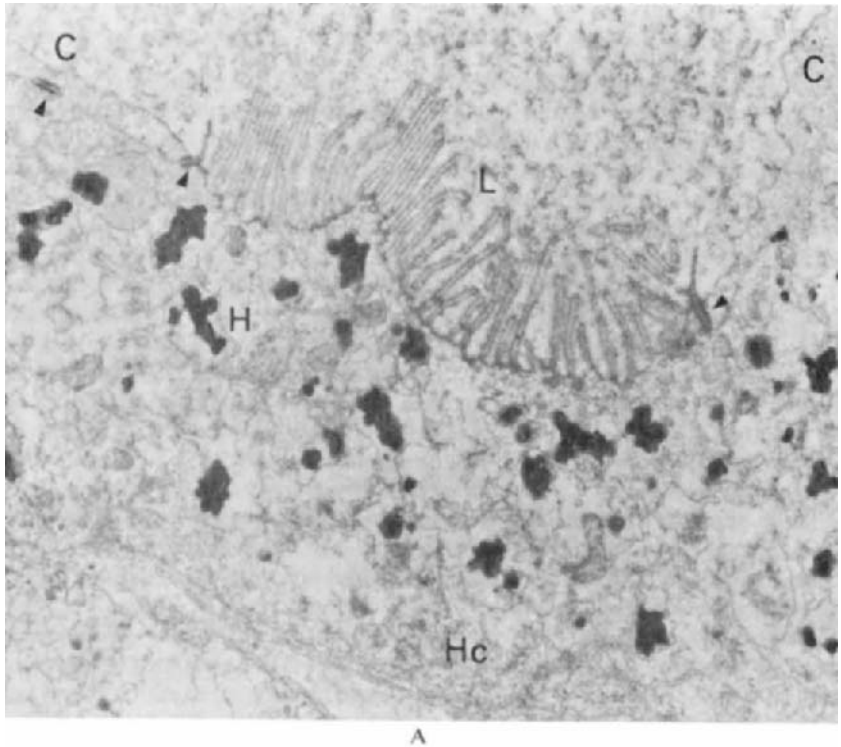



FIG. 9. Diagram showing the regional variation in structure of the anterior alimentary tract of *Diclidophora merlangi*. The main regions of the gut are marked on the left and the epithelial characteristics are described on the right-hand side of the figure. The asterisks indicate the junctions between the different types of epithelia. (Reproduced with permission from Halton and Morris, 1975.)

cells—the prepharyngeal glands which produce basophilic PAS-positive droplets containing protein. The gland cell apices containing secretions form part of the lining of the prepharynx. The lip of the pharynx is covered by an epithelium possessing numerous dense lamellae. The epithelium lining the pharynx itself is in units of up to 2 $\mu$m in length and has numerous short lamellae. The oesophagus is lined with an epithelium possessing lamellae and it closely resembles the gastrodermis, but differs in not having haematin-containing cells. The gland cells associated with the oesophagus are acidophilic and contain electron-lucid, PAS-positive droplets. These develop a crystalline appearance before release into the oesophageal lumen. All these regions of the anterior gut are joined by distinct, septate desmosomes. Rohde (1975) also describes buccal, pharyngeal and post-pharyngeal gland cells in the anterior gut of *Polystomoides*. Gland cells have been recorded from the anterior gut of *Calicotyle kroyeri*, *Entobdella hippoglossi*, *Polystoma intergerrimum* and *Diplozoon paradoxum*, and the secretions seem to be involved either in the digestion of host tissues or in attachment to the host surface (Halton and Jennings, 1965). In *Diclidophora merlangi*, glands discharge secretion into the buccal cavity, prepharynx and oesophagus, and Halton *et al.* (1974) suggest differing functions for these secretions. They believe that the secretion from the buccal glands might play a part in adhesion to the host and possibly function as an anticoagulant, whereas the prepharyngeal gland secretions may be associated with the rapid digestion of erythrocytes which occurs in this region. The role of the oesophageal secretions is not so obvious, but it seems probable that they will contribute to the extracellular digestion of ingested host material. With such vital functions as these it is necessary for secretions to be produced continually throughout the life of the animal. It is not surprising, therefore, that all these gland cells exhibit a merocrine type of secretory activity in which the cells are not completely destroyed.

Ultrastructural studies have increased considerably our understanding of the monogenean gastrodermis. In *Diclidophora merlangi* (Halton *et al.*, 1968), *Protopolystoma xenopi* (Tinsley, 1973), *Polystomoides malayi* and *P. renschii* (Rohde, 1973), *Plectanocotyle gurnadi*, *Diplozoon paradoxum* and *Discocotyle sagittata* (Halton, 1974a), the gastrodermis contains two cell types. In *Polystomoides* the cells are columnar and are completely separated by lateral plasma membranes and joined at the luminal end by septate desmosomes. One of these cells has lamellae on its apical surface. This cell type has an elaborate tubular system as well as many large membrane-bound vacuoles containing an electron-dense material which might be haematin. These nucleated cells contain GER, Golgi complexes, mitochondria and glycogen. The second cell type is nucleated, but is devoid of vesicles containing dense material and does not have apical lamellae. The cytoplasm contains numerous small vacuoles which may be empty or filled with a granular secretion. The lumen of *P. asiaticus* contained intact and disintegrating parts of lamellated cells, suggesting that there is a fairly frequent loss of this cell type from the gastrodermis. However, Rohde (1973) was unable to find evidence of mitotic activity in the epithelium and suggested that replacement might occur by cells from the surrounding parenchyma.

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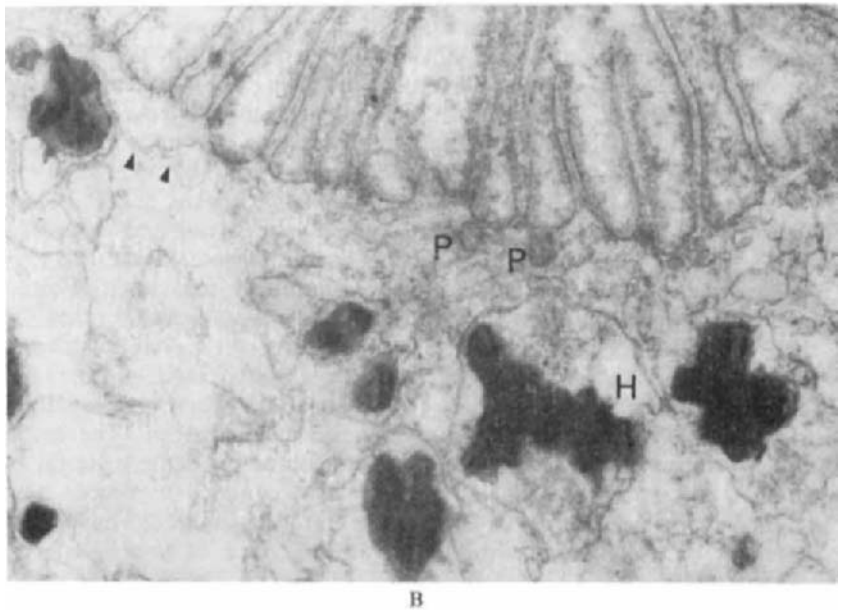



B

FIG. 10A (above). Gastrodermis of *Discocotyle*. The central cell is a haematin cell (**Hc**) bearing lamellae (**L**) and with vesicles containing haematin (**H**). On either side of this cell are overlapping connecting cells (**C**) joined by junctional complexes (arrows). × 11 550. FIG. 10B (below). Detail of haematin cell from the gastrodermis of *Discocotyle*. Haematin residues (**H**) are contained in a ramifying (arrows) system of vesicles. Pinocytotic vesicles (**P**) are derived from the luminal plasma membrane. × 34 000.

In a study of *Diclidophora merlangi*, Halton has not only described the ultrastructure of the gastrodermis (Halton *et al.*, 1968) but has given an account of the intracellular changes occurring in relation to the digestion of haemoglobin (Halton, 1974b, 1975). The gastrodermis of this species contains nucleated haematin cells which have a lamellated apical membrane. These cells occur singly and may lie close together, but never immediately adjacent or widely dispersed. The haematin cells are linked by what Halton *et al.* (1968) term connecting cells. These are thin and flat and are connected to the haematin cells by septate desmosomes, but the connecting cells themselves are thought to form a syncytium. The cells have lamellae on their luminal surface and the cytoplasm contains a small amount of GER and occasional Golgi complexes. Connecting cells may be folded or branched and frequently overlap the haematin cells. (See Fig. 10A of *Discocotyle*, which closely resembles *Diclidophora*.)

A conspicuous feature of the haematin cells is the presence in the cytoplasm of an anastomosing system of irregularly shaped, membrane-bound channels. These have expanded portions which contain haemoglobin or haematin. Most of the cytoplasm is occupied by this system, but adjacent to the basal nucleus are strands of GER, mitochondria and Golgi complexes (Halton, 1974b). By a study of starved and fed *Diclidophora*, Halton (1975) has investigated the role of these channels in the intracellular digestion of haemoglobin (see Figs 10B, 11). Haemoglobin present in the lumen of the caecum is endocytosed into small vesicles derived from the apical plasma membrane. It is thought that these vesicles fuse with the intracellular system of channels so that the haemoglobin becomes transferred into this system. Vesicles, derived from the Golgi complexes and containing hydrolytic enzymes, are thought to fuse with the channels so that hydrolysis of the host's haemoglobin occurs within the channels. The haematin residues are exocytosed into the the caecal lumen when the apical ends of the channels fuse with the plasma membrane of the cell. Thus, in this species, elimination of haematin does not involve the shedding or disruption of the haematin cell as suggested by earlier authors (see Halton and Jennings, 1965; Jennings, 1968) but is a continuous process which goes on simultaneously with the process of absorption and intracellular digestion. The haematin cell therefore shows signs of compartmentalisation in which the basal portion of the cell is involved in the synthesis and packaging of digestive enzymes, whereas the apical portion has become specialised for the intake of exogenous material, intracellular digestion involving a lysosomal sequence and the elimination of undigested residues.

It is possible that this pattern may be representative of gastrodermal function in most of the Polyopisthocotylea. A brief account of the caecal structure of *Calicotyle kroyeri* (Halton and Stranock, 1974) suggests that a slightly different situation may exist in the Monopisthocotylea. In *Calicotyle* the gastrodermis contains one type of cell which functions in the uptake and digestion of fish mucus. The cell cytoplasm contains little GER, few Golgi complexes, and a basal nucleus. The apical plasma membrane forms vesicles and a complex network of tubules which function in endocytosis and intracellular digestion. The main difference is in the absence of connecting cells. The function of these

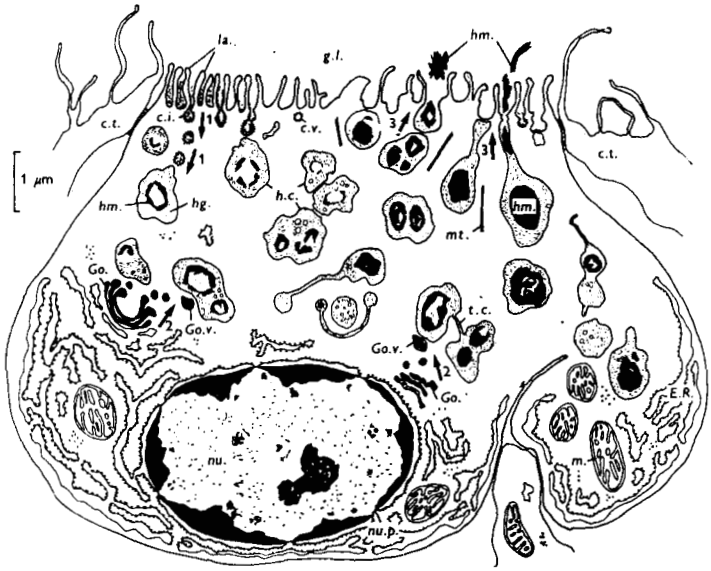



FIG. 11. The proposed pathway for intracellular digestion of haemoglobin in the haematin cell of *Diclidophora merlangi*. The routes are indicated by arrows: **1**, endocytosis of haemoglobin; **2**, the transfer of vesicles containing enzymes from the Golgi complexes to the haematin channels; **3**, exocytosis of residual haematin at the cell surface. **ci**, coated invaginations; **ct**, connecting tissue syncytium; **cv**, coated vesicle; **ER**, granular endoplasmic reticulum; **gl**, gut lumen; **Go**, Golgi stacks; **Go.v**, Golgi vesicle; **hc**, haematin channel; **hg**, haemoglobin; **hm**, haematin; **la**, lamellae; **m**, mitochondrion; **mt**, microtubule; **nu**, nucleus; **nu.p**, nuclear pore; **tc**, tubular connection. (Reproduced with permission from Halton, 1975.)

cells in the Polyopisthocotylean gastrodermis is far from clear. They may give mechanical stability to the gastrodermis and support the haematin cells or they might have a function in the absorption of soluble nutrients from the caecal lumen.

(a) *Histochemical studies.* The earlier studies demonstrating the presence of haemoglobin and haematin in the caecal lumen and gastrodermis have been reviewed by Halton and Jennings (1965), Jennings (1968) and Erasmus (1972). Halton (1967b) has shown that alkaline phosphatase activity occurs in the gastrodermis of seven genera studied (*Entobdella*, *Calicotyle*, *Polystomum*, *Diplozoon*, *Discocotyle*, *Diclidophora* and *Octodactylus*), whereas acid phosphatase activity is absent. Nonspecific esterase activity was demonstrated in the gastrodermis of *Calicotyle* and *Polystomum* and thiaminepyrophosphatase in the gastrodermis of *Diclidophora* (Halton, 1975).

There is a general impression that extracorporeal digestion may take place by the loss of enzymatic material through the buccal cavity onto the host tissues. Gelatinolytic activity of this type has been demonstrated in extracts of the anterior end of *Polystoma intergerrimum* (Halton and Jennings, 1965) and *Diclidophora merlangi* (Halton *et al.*, 1974). The apparent absence of

hydrolases from the gastrodermis of the Polyopisthocotylea is rather anomalous in the light of the ultrastructural findings, which have unequivocally demonstrated the presence of intracellular digestion associated with lysosomal activity.

These new studies indicate quite clearly that considerable differences exist, both at a cellular and subcellular level, between the gut of digeneans and monogeneans. In many ways our understanding of gastrodermal function is more clear for the monogeneans than for the digeneans. It is hoped that the application of modern cytological ideas and practices will further clarify the many puzzles which remain. There are many contradictions. Some species have a cellular gastrodermis whereas in others it is syncytial. The luminal surface of the caecum of some flukes bears lamellae whereas in others it is microvillous. Several larval stages possess a well developed gastrodermis which is apparently nonfunctional.

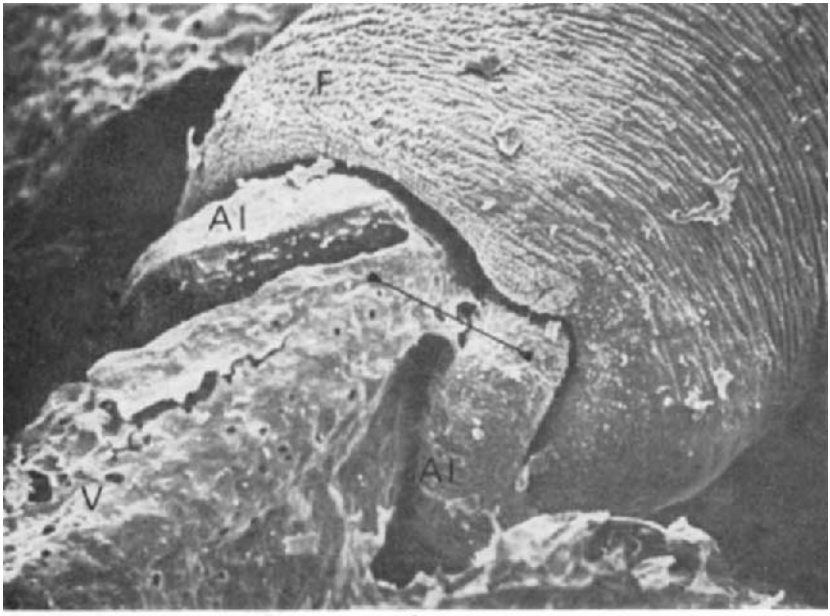
The trematodes occupy a wide variety of habitats and consequently ingest a considerable range of food which might consist of cells or host secretions and which can be solid or liquid in consistency.

The studies described indicate that the trematode gut has evolved to give many variations, although the diversity can only be truly appreciated at an ultrastructural level.

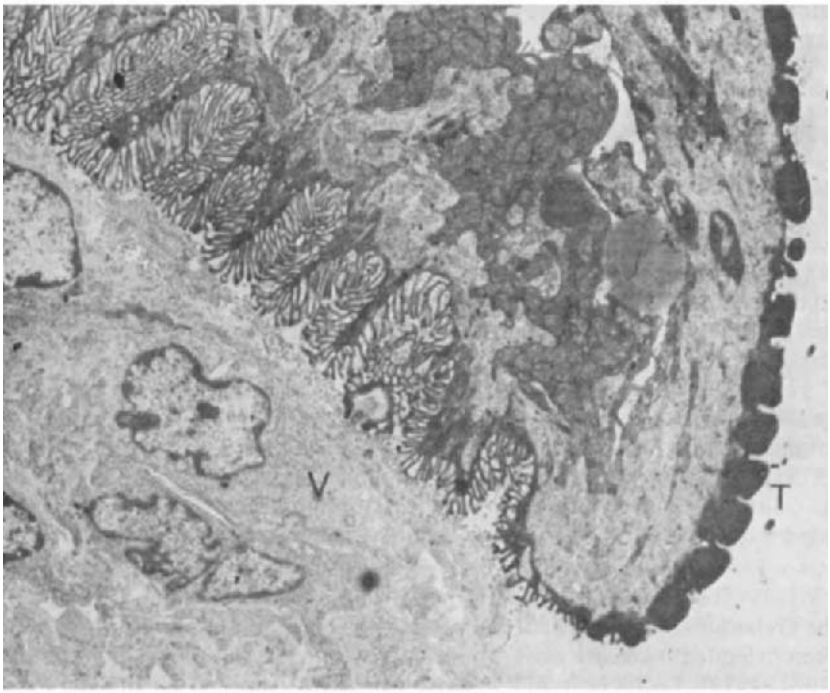
## III. The Tegument

Since the statement by Threadgold (1963a, b), describing the cytoplasmic nature of the digenean tegument, there has been a considerable extension of our knowledge of this investing layer. The digenean pattern of an external layer of cytoplasm joined by cytoplasmic bridges with nucleated masses of cytoplasm lying internal to the body wall musculature, has been shown to occur also in the other major divisions of the Trematoda. The terminology of this surface layer has had a varied history and although "tegument" is generally accepted, the range and validity of the alternatives which might be used have been discussed by Lee (1966), Smyth (1972, 1973) and Lumsden (1975).

The biological significance of this cytoplasmic interface between the parasite and its external environment has attracted considerable attention and the original studies have been extended to the Monogenea (see reviews by Lyons, 1973 and Rohde, 1975) and the Aspidogastrea (Rohde, 1972). Regional specialisation of the surface (see Fig. 12) where morphological change can be related to functions such as secretion and absorption has been shown to occur not only in the digenean strigeids (Erasmus and Öhman, 1963, 1965; Erasmus, 1967, 1968, 1969a, b, c 1970a, b, c) but also in all the other major divisions of the Trematoda. Our knowledge of sense organ morphology and distribution has been extended by many workers and reviewed by Lyons (1972, 1973), Rohde (1972, 1975), Lee (1972) and Brooker (1972). In spite of the morphological detail now available the precise function of these structures is far from clear. The trematodes are characterised by having a variety of larval stages in the life-cycle, and the nature of the external covering of these various stages and the changes which occur throughout the life-cycle have been described in




A




B

FIG. 12A (above). Stereoscan photograph of *Apatemon gracilis minor* showing the adhesive organ lobes (**Al**) protruding from the fore-body cup (**F**) and enclosing a villus (**V**) of the host (duck) intestine. The arrowed line indicates the plane of the section shown in B. × 1380.
FIG. 12B (below). Transmission electronmicrograph of a section through the region marked on the stereoscan photograph. The normal tegument (**T**) is replaced by lamellate and highly infolded tegument on the inner face of the adhesive organ lobe which comes into contact with host tissue. **V**, villus. × 3000.

several species (see reviews by Lyons, 1972, 1973; Rohde, 1972, 1975; Lee, 1972; Lumsden 1975) although the most detailed information is available on *Schistosoma* (see Hockley, 1973; Stein and Lumsden, 1973; Stirewalt, 1974). Nearly all of the early descriptions were based on the examination of thin sections in the transmission electronmicroscope (TEM) but more recently this rather restricted viewpoint is being supplemented by scanning observations of entire specimens and these integrated studies have produced a more realistic impression of this outer surface.

Inevitably, the basic morphological studies were linked with and followed by investigations of a different sort (histochemical, immunological and uptake studies) intended to clarify the more dynamic aspects of the tegument. The functional complexity of the tegument is clearly indicated in the review of cytochemical studies by Lumsden (1975) and of uptake studies and membrane transport by Pappas and Read (1975). In his review of the early work on the digenean tegument, Erasmus (1972) suggested that a detailed study of the characteristics of the external plasma membrane would provide a valuable basis for a realistic interpretation of function in the limiting external surface of trematodes. Lumsden (1975) considers in considerable detail the structure and histochemistry of this external layer, emphasising particularly the outer glycocalyx, and discusses its possible role in permeability control and ionic regulation, protection of the parasite against host enzymes, involvement in antibody/antigen reactions and surface inhibition or inactivation of host enzymes. Pappas and Read (1975), reviewing membrane transport in helminths, suggest that although this glycocalyx might be regarded as the "host–parasite interface", the concept of "interface" implies the existence of a surface and is unrealistic in that the processes of digestion and absorption of host material probably occur in a space distal to, but including, the outer plasma membrane in all its complexity. They propose the term "host–parasite interfacial space" as being more meaningful in the understanding of absorptive processes occurring at this surface. Ideas such as these emphasise the functional continuity which exists between parasites having a cytoplasmic tegument and the host environment, and link the more morphological concepts with the functional ideas of membrane transport.

The interrelationship between tegumental structure and ion transport and osmoregulation has been discussed by Mettrick and Podesta (1974), who suggest that the tegument is morphologically polarised containing a variety of membrane-associated transport systems. Unfortunately, at present, there is no information concerning the relationship between the distribution of inorganic ions and the membrane systems of the tegument. Histochemical techniques for the study of inorganic substances are very unreliable and new techniques such as the X-ray analysis in the TEM of frozen sections of specimens quenched in liquid nitrogen (see Davies and Erasmus, 1973 and Erasmus, 1974, 1975) might provide valuable information. An example of such an analysis is given in Fig. 7B. This indicates the presence of sodium, phosphorus, sulphur, chlorine and potassium in the spined tegumental cytoplasm of female *Schistosoma mansoni*. The prominent S (sulphur) peak is absent from the unspined areas, suggesting a link between S and the tegumental spines. These techniques

are currently being developed, and by the analysis of areas 50 nm in diameter the variation in distribution and concentration of elements throughout the different regions of the tegument can be assessed.

Our desire to understand the nature and functioning of this outer limiting surface has stimulated the interest of many workers over the last 10 years, and the considerable progress achieved is evinced by the detailed reviews which have appeared since 1972. The current interest is to link function with morphology, and it is hoped that the more interdisciplinary studies will clarify many of the problems confounding us at present.

## Acknowledgements

I am indebted to Dr C. J. Blake for permission to use one of his unpublished electron micrographs (Fig. 7). I am also grateful to Mrs C. Winters and Mr T. W. Davies for their highly skilled assistance in preparing some of the material used to illustrate this paper.

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# Brugian Filariasis: Epidemiological and Experimental Studies

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## I. INTRODUCTION

### A. GENERAL

Filarial nematodes are arthropod-borne parasites which sometimes cause serious disease in man and animals. These parasites have been studied by a wide range of scientists ranging from biochemists and electron microscopists to taxonomists and epidemiologists. Work on the medically important filarial worms can be divided into two broad categories, "research and development" and "epidemiology and control". At the London School of Hygiene and Tropical Medicine, we have had the opportunity to discuss filariasis with many public health workers from countries where it is endemic and we recognise the need for a much closer contact between the experimental and applied scientists. Our expertise is based on experimental filariasis and it is our objective to summarise the status of research and to emphasise its relevance to human disease. We have made a special effort to identify problems which impede the development of new control techniques and have included some of our unpublished work when it is relevant to their solution. The review is concluded with a personal evaluation of current control programmes and the prospects for the future. We hope that our review will be of interest to experimental parasitologists, public health personnel and teachers and students of tropical medicine and parasitology.

The most important filarial worms of man are *Onchocerca volvulus*, the cause of river-blindness and severe skin disease, and *Wuchereria bancrofti*, the cause of tropical elephantiasis and hydrocoel. Bancroftian filariasis is found throughout the tropical world while onchocerciasis is limited to Africa and Central and South America. The third serious pathogen of man is *Brugia malayi* and its distribution is limited to Asia (Fig. 1).

Filarial nematodes are noted for their narrow specificity for vertebrate hosts, and *O. volvulus* and *W. bancrofti* infect only man. However, *B. malayi* was successfully introduced into a variety of laboratory hosts some 20 years ago. The discovery of these animal systems has facilitated the expansion of research on filaria–vector relationships and vertebrate pathology, immunology and chemotherapy. It is significant that the life-cycle of *Brugia* spp. in animals mimics that of *Brugia* and *Wuchereria* in man and advances in laboratory studies have led to a better understanding of the human diseases.

In 1947, Stoll estimated that there were at least 189 million people with *W. bancrofti* and *B. malayi* and in the same year Hewitt *et al.* reported the discovery of diethylcarbamazine (DEC) which is still the only antifilarial drug available for use in man. Despite the availability of this drug for 29 years, the World

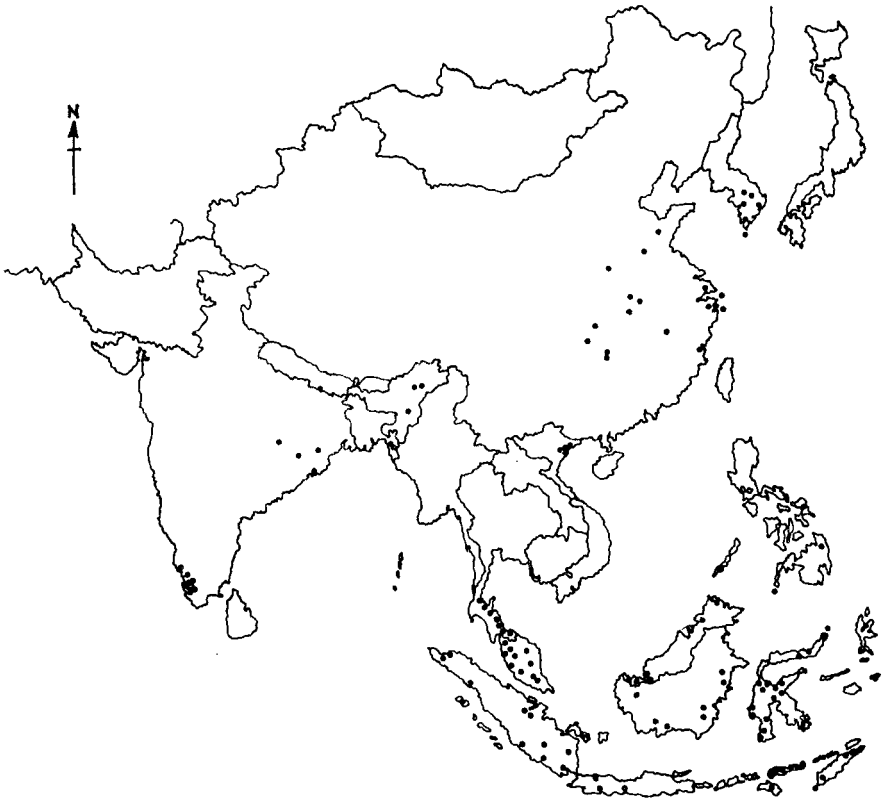


FIG. 1. Distribution of *Brugia malayi* in Asia.

Health Organization estimated that there were at least 250 million cases of filariasis in 1974. It must be concluded that DEC and laboratory studies have had little impact on global prevalence.

### B. TAXONOMIC NOTE

Brugian filariasis was first recognised as clinically distinct from Bancroftian filariasis by Lichtenstein in 1927. These observations were supported by Brug (1927, 1929), who showed that the microfilariae associated with Lichtenstein's disease were morphologically distinct from *W. bancrofti* and deserved a special taxon which he called *Filaria malayi*. The adult worms were not described until 1940 when Rao and Maplestone established the species *Wuchereria malayi*. Buckley (1958b) compared the morphology of *W. bancrofti* with *W. malayi* and two newly described animal filariids, *Wuchereria pahangi* and *Wuchereria patei*, and concluded that *malayi*, *pahangi* and *patei* were closely related. He placed them in a new genus called *Brugia* leaving *Wuchereria* as a monospecific genus containing only *bancrofti*. Perhaps Buckley should be

TABLE I

*The species, natural mammalian hosts, sites of infection and geographic distribution of the genus* Brugia

| Species | Natural mammalian hosts | Site of infection | Distribution | Reference |
|---|---|---|---|---|
| *Brugia malayi* (Brug, 1927) Buckley, 1958 | Man (*Homo sapiens*)<br>Long-tailed macaque (*Macaca irus*)<br>Dusky leaf-monkey (*Presbytis obscurus*)<br>Banded leaf-monkey (*Presbytis melalophos*)<br>Silvered leaf-monkey (*Presbytis cristatus*)<br>Cat (*Felis catus*)<br>Wild cat (*Felis bengalensis*)<br>Wild cat (*Felis planiceps*)<br>Civet cat (*Paradoxurus hermaphroditus*)<br>Civet cat (*Arctogalidia trivirgata*)<br>Pangolin (*Manis javanica*) | Lymphatics and lymph nodes | Asia, Fig. 1. | Edeson and Wilson (1964) |
| *Brugia pahangi* (Buckley and Edeson, 1956) Buckley, 1958 | Dusky leaf-monkey (*P. obscurus*)<br>Slow loris (*Nycticebus coucang*)<br>Cat (*F. catus*)<br>Dog (*Canis familiaris*)<br>Tiger (*Panthera tigris*)<br>Wild cat (*F. bengalensis*)<br>Wild cat (*F. planiceps*)<br>Civet cat (*P. hermaphroditus*)<br>Civet cat (*A. trivirgata*)<br>Civet cat (*Viverra zibetha*)<br>Civet cat (*Arctictus binturong*) | Lymphatics and lymph nodes | West Malaysia | Edeson and Wilson (1964) |

| | | | | |
|---|---|---|---|---|
| | Otter (*Lutra sumatrana*)<br>Pangolin (*M. javanica*)<br>Moon-rat (*Echinosorex gymnurus*)<br>Giant-squirrel (*Ratufa bicolor*) | | | |
| *Brugia patei* (Buckley, Nelson and Heisch, 1958) Buckley, 1958 | Dog (*C. familiaris*)<br>Cat (*F. catus*)<br>Genet cat (*Genetta tigrina*)<br>Bush baby (*Galago crassicaudata*) | Lymphatics and lymph nodes | Kenya | Nelson (1965) |
| *Brugia buckleyi* Dissanaike and Paramananthan 1961 | Ceylon hare (*Lepus nigricollis singhala*) | Heart, pulmonary arteries, hepatic veins, vena cava | Sri Lanka | Dissaniake and Paramananthan (1962) |
| *Brugia ceylonensis* Jayewardene, 1962 | Dog (*C. familiaris*) | Lymph nodes and associated tissue | Sri Lanka | Jayewardene (1962) |
| *Brugia beaveri* Ash and Little, 1964 | Raccoon (*Procyon lotor*) | Lymph nodes, "skin and body soakings" | U.S.A. (Louisiana) | Ash and Little (1964) |
| *Brugia guyanensis* Orihel, 1964 | Coatimundi (*Nasua nasua vittata*) | Lymphatics | Guyana | Orihel (1964) |
| *Brugia tupaiae* Orihel, 1966 | Tree shrew (*Tupaia glis*)<br>Tree shrew (*Tupaia tana*) | Lymphatics | Malaysia, Thailand, North Borneo | Orihel (1966, 1967) |

congratulated for naming the genus after Brug rather than Lichtenstein for the sake of pronunciation but Lichtenstein should be recognised for the astute clinical observations which eventually led to the identification of the new human parasite.

In addition to *B. malayi* there may be another species of *Brugia* infecting man on the islands of Timor, Flores, Alor and Roti (Wheeling *et al.*, 1975). The initial separation of this new form from *B. malayi* was made by David and Edeson (1965) based on differences in the morphological characteristics and staining reactions of the microfilariae, and they called this variant the "Timor microfilaria". The adult worm has recently been reared in laboratory animals and preliminary morphological studies indicate that this is a new species (Dennis, pers. comm.).

During the 1950s it was still believed that human filarial worms were host-specific to man, but in the course of extensive epidemiological investigations in West Malaysia a variety of alternative hosts for *B. malayi* were found. It was also found that animals are infected with their own species of *Brugia* and today eight species are recognised (Table I). All of these species have similar life cycles in mosquitoes and vertebrates and are parasites of the lymphatic system except *Brugia buckleyi*, which lives in the heart and blood vessels of the Ceylon hare. Dissanaike and Paramananthan (1962) suggested that this species should be placed in a new subgenus—*Brugiella*. The differences between *B. buckleyi* and the other members of the genus *Brugia* are so pronounced that a new genus should be erected to contain this single species.

### C. LIFE-CYCLE

This generalised account of the life-cycle applies to *Brugia* spp. and *W. bancrofti*. There are specific differences between these genera and between the species of *Brugia* which are mentioned later when deemed important.

Filarial worms, like other nematodes, have five developmental stages in their life-cycles. The adults or fifth-stage worms infect the lumina of the lymphatics where they may live for years. They have a thread-like appearance and male *B. malayi* measure $0{\cdot}1 \times 22{\cdot}0$ mm while females measure $0{\cdot}15 \times 48{\cdot}0$ mm. After mating, the females continuously lay microfilariae or first-stage larvae which measure only $6 \times 210$ $\mu$m. The microfilariae migrate from the lymphatics to the blood stream. If they are ingested by blood-feeding mosquitoes they migrate to the thoracic muscles and moult twice to the infective or third larval stage. This process usually takes 1–2 weeks and the larvae increase their size to $0{\cdot}03 \times 1{\cdot}5$ mm. When the mosquito bites the vertebrate host, the infective larvae migrate from the skin to the lymphatics where they moult twice to become adolescent and finally, reproducing adults.

## II. HUMAN FILARIASIS

### A. PERIODIC AND SUBPERIODIC *BRUGIA MALAYI*

Microfilariae have a unique circadian periodicity and are found in the peripheral circulation during certain periods of the day. Mosquitoes, the

vectors of *Brugia*, also have a circadian rhythm in which they bite at specific periods of the day. The highest concentration of microfilariae in the peripheral blood occurs during the period that the local mosquito vector is most actively feeding.

There are two well-defined strains of *B. malayi* which differ in their microfilarial periodicity patterns (Fig. 2). The nocturnally subperiodic strain is found in the dense swamp forest where its mosquito vectors prefer to feed at night, but also bite during the day. The nocturnally periodic strain of *B. malayi* is found in the open plains where the vectors feed only at night. The correlation between peak peripheral microfilaraemia and peak biting time suggests that filarial nematodes have adapted their periodicity patterns to vector periodicity patterns to facilitate their transmission by mosquitoes (Wharton, 1963).

Turner and Edeson (1957) first described the nocturnally periodic and subperiodic strains of *B. malayi* in man, but the periodicity patterns of each form are variable in different host species. For example, the strain of *B. malayi* which is markedly nocturnally periodic in man becomes subperiodic in cats, while the strain which is subperiodic in both man and the cat is nocturnally

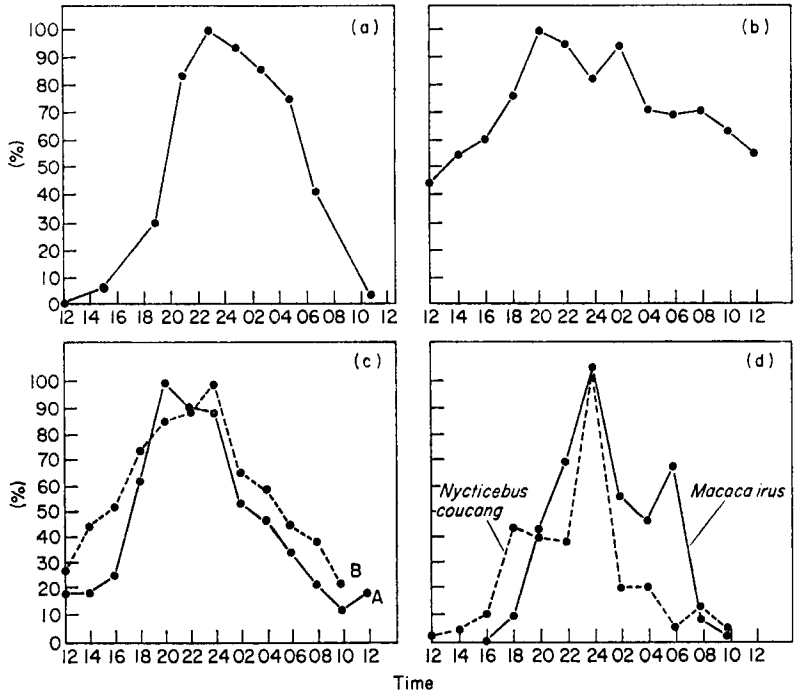


FIG. 2. Circadian periodicity of the microfilariae of *B. malayi* in different hosts expressed as a proportion of the maximum parasitaemia. (a) Nocturnally periodic strain in man (Turner and Edeson, 1957). (b) Nocturnally subperiodic strain in man (Turner and Edeson, 1957). (c) Subperiodic strain in cats ("A" from Edeson, 1959; "B" from Burren, 1972). (d) Subperiodic strain in two species of monkeys (Edeson, 1959).

periodic in *Macaca irus* and *Nycticebus coucang* (Edeson, 1959; Laing, 1961; Laing *et al.*, 1961; Dondero *et al.*, 1972; Guptavanij *et al.*, 1971c; Burren, 1972). Figure 2 shows the periodicity patterns of these two forms in man and other hosts.

In addition to the two basic strains of *B. malayi*, a third form has been described which exhibits a diurnally subperiodic pattern (Cabrera and Rozeboom, 1956; Dondero *et al.*, 1971; Guptavanij *et al.*, 1971a). Nothing is known about other biologic characteristics of this form. Since it is sympatric with nocturnally subperiodic *B. malayi*, it may simply be a variant of this strain.

### B. MECHANISMS CONTROLLING PERIODICITY

Edeson *et al.* (1957) applied the techniques which had been used to study the mechanism responsible for the periodicity of *Wuchereria bancrofti* (Hawking, 1967) to patients infected with nocturnally subperiodic *B. malayi*. If volunteer carriers breathed pure oxygen at night there was a 91 % reduction in the number of microfilariae in the peripheral circulation. During the day the reduction was 47%. Working with the periodic strain, Hawking *et al.* (1966) found that after breathing oxygen for 13 min at night, the microfilarial counts fell by only 40%. Hawking and Gammage (1968) found that oxygen produced a dramatic decline in the number of microfilariae circulating at night in *Macaca mulatta* infected with nocturnally subperiodic *B. malayi*. Burren (1972) found a similar decline in the number of microfilariae in the blood of cats when they breathed oxygen in the late evening.

In humans infected with periodic *B. malayi*, hypoxia, hyperventilation, altered carbon dioxide levels or exercise did not affect the density of microfilariae as dramatically as did inhalation of oxygen (Edeson *et al.*, 1957; Hawking *et al.*, 1966). Hawking proposed that the appearance of microfilariae in the peripheral circulation is controlled by the difference in oxygen tensions between arterial and venous blood in the lungs. Whilst there is no direct evidence for *Brugia* spp., it has been shown that other nocturnally periodic microfilariae are in the arterioles of the lung when they cannot be detected in peripheral circulation. Hawking *et al.* (1966) quote various sources to illustrate the oxygen content of the arterial and venous systems in sleeping and active subjects after exposure to abnormal oxygen-inert gas mixtures or exercise. They suggest that if the difference in venous-arterial oxygen tension is under 50 mmHg, microfilariae will leave the lung for the general circulation, but if the difference is greater than 53 mmHg they will accumulate in the lungs. This hypothesis is very attractive. The lowering of the venous-arterial oxygen tension difference at night is due to a decrease in the amount of oxygen in the arterial system because of shallow breathing and a rise in the venous oxygen tension occurs because less oxygen is consumed during rest than when the subject is awake and active.

### C. DIFFERENTIATION OF PERIODIC AND SUBPERIODIC *BRUGIA MALAYI*

There are no reliable morphological differences between the two strains of *B. malayi*. However, the strains can easily be separated by the proportion of

microfilariae which cast their sheaths while being stained with Giemsa; large proportions of periodic microfilariae exsheath compared to subperiodic microfilariae (Guptavanij and Harinasuta, 1971; Sivanandam and Dondero, 1972). The subperiodic and periodic strains of *B. malayi* can also be differentiated by xenodiagnosis. *Anopheles barbirostris*, *An. campestris* and *An. donaldi* have high indices of experimental infection when infected with periodic *B. malayi* and low indices when infected with subperiodic *B. malayi*. The indices of infection are the reverse in *Mansonia bonneae*, *M. dives* and *M. annulata* (Wharton, 1962).

### D. NATURAL VECTORS OF PERIODIC *BRUGIA MALAYI*

The natural vectors of the two strains of *B. malayi* are generally different species which have allopatric distributions. The periodic strain of *B. malayi* is found in the agricultural areas with its anopheline and aedine vectors while the subperiodic strain is found in the forest with its mansonoid vectors. Generally the anopheline vectors of periodic *B. malayi* do not invade nearby forests nor do the vectors of the subperiodic strain leave the forest for agricultural areas. The obvious exceptions to this rule are *Mansonia uniformis*, *M. annulifera* and *M. indiana*, which transmit both strains and are found in both areas. The vectors of *Brugia* spp. have been listed on several occasions (WHO, 1974; Ramalingam *et al.*, 1968; Chow, 1973).

The larval stages of *Mansonia* are unique among the culicidae because they attach to water plants by their siphon to obtain oxygen. Each *Mansonia* spp. has a preference for a variety of plants, but most of the important vectors attach to water lettuce, *Pistia stratiotes*. These plants are widely distributed in open swamps, ditches, tanks and ponds. The ponds often serve as water reservoirs or as soakage pits to process coconut fibre and the plants may be harvested as pig fodder. In endemic areas of India, Sri Lanka and West Malaysia small plant-covered ponds are found in profusion where they support the development of large populations of *Mansonia* which transmit periodic *B. malayi*.

The anopheline vectors are associated with clean sources of water. Although the anopheline larvae are found in ditches and wells, they prefer the rice fields around villages where they breed in large numbers. *Aedes togoi*, a vector in Japan, Korea and China, breeds predominantly in rock holes containing brackish water and in rain water in artificial containers (Chow, 1973; Kim *et al.*, 1973a).

In regions where nocturnally periodic *B. malayi* occurs, both the number and diversity of wild animals is limited by human agricultural activities, and the night-biting mosquitoes readily feed on domestic animals such as cows, water buffalo, goats, cats and dogs. Under experimental conditions, periodic *B. malayi* produces patent infections in cats, *Macaca mulatta* and *Presbytis obscurus* but is seldom found in animals in nature (Laing *et al.*, 1961). It is generally thought that nocturnally periodic *B. malayi* is not a zoonosis and it is possible that the few infected animals are the victims of infection derived from man. Figure 3 shows the relationship between different mammalian hosts.

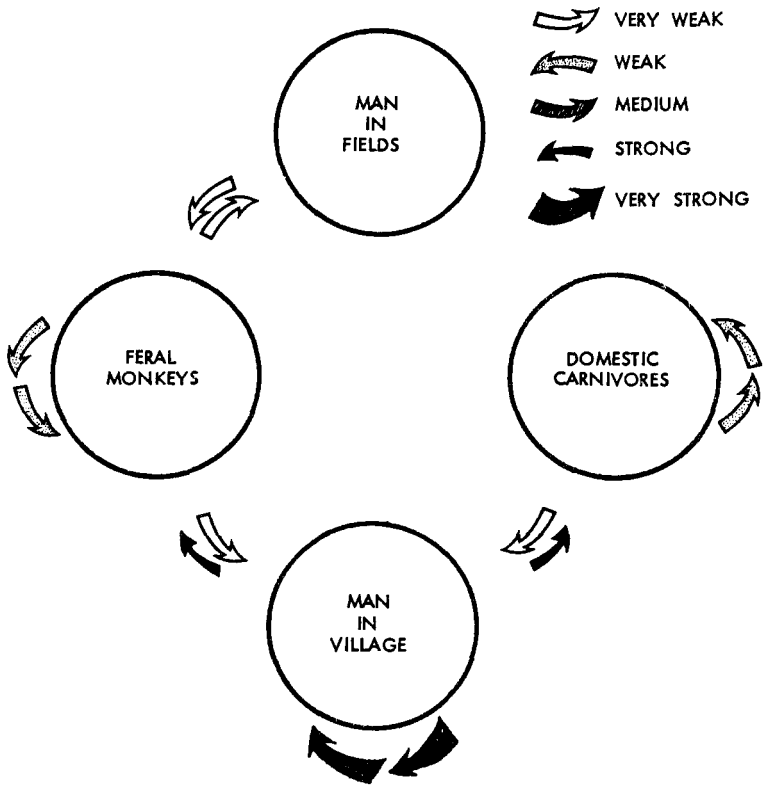


FIG. 3. The probable feral and domestic cycles of nocturnally periodic *B. malayi*.

### E. ZOONOTIC SUBPERIODIC *BRUGIA MALAYI*

Nocturnally subperiodic *B. malayi* is common in wild monkeys (Table I) and this led to the assumption that it is zoonotic. Before deciding if an infection is a zoonosis, it is vital to know the susceptibility of man and animals to infection, the susceptibility and host preferences of the mosquitoes and the frequency of transmission of infective worms between animals and man.

There have been three attempts to transmit *Brugia* spp. from animals to man. Low densities of microfilariae were found in the blood in one of three men infected with *B. pahangi* and one of six men with *B. malayi* (Buckley, 1958a; Edeson *et al.*, 1960; Dondero *et al.*, 1972). However, all of the subjects experienced clinical signs and symptoms of filariasis indicating that they were infected. It is possible that the subjects who failed to become patent had low, undetectable, densities of microfilariae or unisexual or adolescent worms.

Nocturnally subperiodic *B. malayi* is limited to foci in the swamp forests of West Malaysia, Thailand, Sabah, Sarawak, South Sumatra, Java, Philippines and Vietnam (Hawking, 1973), where man and his domestic animals are

surrounded by virgin forest with wild animals and mosquitoes. The villages are set in small clearings and rice is often cultivated around their perimeters. Man and his domestic animals serve as hosts for mosquitoes which migrate in and out of the surrounding forest. Men enter the forest to fish, hunt, tap rubber, collect timber and rattans or to communicate with neighbouring villages. In general, the children are confined to the houses and the women remain in the village except to tend the rice paddies or to visit the men employed in the forest.

*Mansonia* mosquitoes, the vectors of subperiodic *B. malayi*, feed on a variety of animal hosts including man. From live bait catches and bloodmeal identification of mosquitoes captured inside and outside houses, Wharton (1962) concluded that *Mansonia* preferred to bite cattle followed by humans and goats, and then pigs, dogs, cats and fowl.

The majority of *Mansonia* feed outdoors and never enter the houses. Wharton (1962) collected mosquitoes attracted to man during the night and found the outside biting rate to be two to three and a half times the indoor rate, depending on the species of *Mansonia*. As the people would normally be sleeping in their bedrooms between 22.00 and 06.00 hours, mosquitoes biting outdoors during this time would have little importance in the transmission of *B. malayi* to humans. However, *Mansonia* do enter houses to feed and may spend up to 20 min resting on the walls before leaving. Although they do not like houses and prefer to bite outdoors, filarial transmission probably occurs in the sleeping rooms as infants are often infected (Wilson, 1961).

It is impossible to differentiate the mosquito stages of *B. malayi* of monkey or human origin and it is difficult to provide substantial proof that the infection is a zoonosis. However, some indirect evidence is available. *Dirofilaria immitis* and *D. repens* are common parasites of domestic carnivores while *D. magnilarvata* infects forest monkeys (Wharton, 1962). Infective larvae of each of these species have been recovered from *Mansonia* vectors of *B. malayi* attracted to human bait, thus proving that these mosquitoes bite monkeys, cats and dogs in addition to man (Wharton, 1962). As monkeys, cats, dogs and other carnivores are also hosts for *B. malayi*, this parasite must also be transmitted from animal to man.

The significance of the animal reservoir was also shown in experimental control schemes in which the human source of infection to mosquitoes was removed with drugs, residual insecticides or both. The people of one village were treated with DEC, which reduced both the infection rate and the microfilarial density (Wharton *et al.*, 1958). The reduced infectivity of the human reservoir was reflected in the vector mosquitoes as only 3–4% of *M. dives/bonneae* that fed on volunteers became infected compared with the 30% which became infected before treatment. Despite this, the infection rate of the mosquitoes coming to bite man remained unchanged. These mosquitoes could have been infected with *B. malayi* or *B. pahangi* because they are sympatric and their infective larvae are morphologically indistinguishable. The sources of mosquito infections must have been the domestic carnivore and wild monkey populations. Similar results were obtained in a second village which was sprayed with residual insecticides (Wharton and

Santa Maria, 1958). Although 80% of the females which fed on man died from the insecticide, there was no obvious reduction in the intensity of transmission. In a third village the two types of control were applied at the same time (Wharton, 1962), but the intensity of transmission of infective larvae to man was only halved.

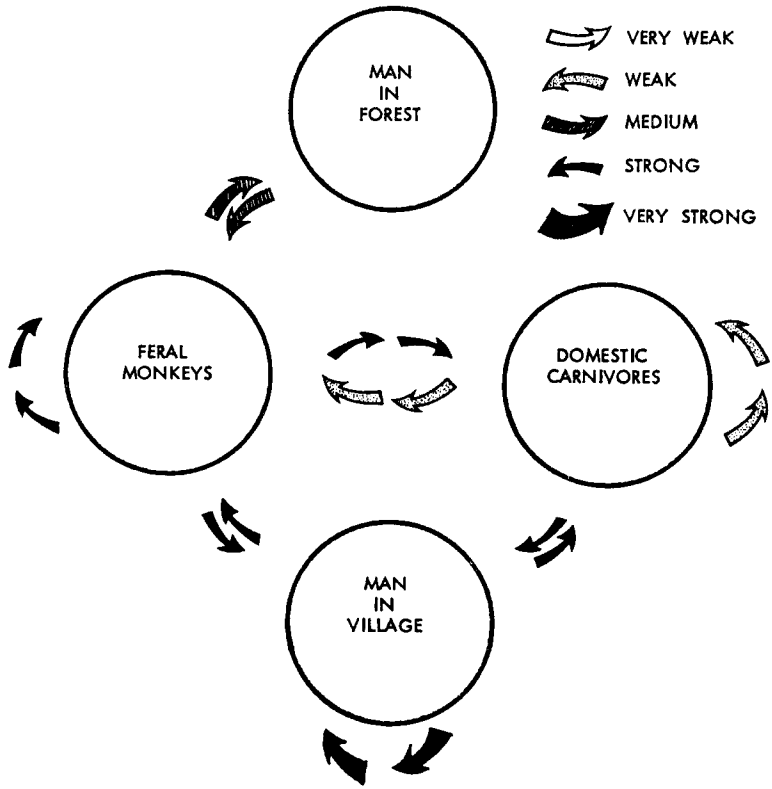


FIG. 4. The probable feral and domestic cycles of nocturnally subperiodic *B. malayi*.

The relative importance of domestic and forest reservoirs is not known, but it is likely that transmission to man would occur with greater frequency from domestic animals. If this hypothesis is correct, a reduction in domestic transmission could be produced by treating the cats and dogs with an effective anthelmintic. This would be reflected in the decreased prevalence of *Brugia* in youngsters and women who seldom venture beyond the village boundaries rather than in the men whose occupation necessitates their continued entry into the forest. A diagrammatic representation of the relationship between subperiodic *B. malayi* and its various hosts is shown in Fig. 4.

### F. DIFFERENTIATION BETWEEN SUBPERIODIC *BRUGIA MALAYI* AND *B. PAHANGI*

Although it is possible to infect human volunteers with both *B. pahangi* and subperiodic *B. malayi* (Buckley, 1958a; Edeson *et al.*, 1960; Dondero *et al.*, 1972) only *B. malayi* has been recognised in natural human infections. However, *B. pahangi* and subperiodic *B. malayi* are sympatric in West Malaysia where it is possible that man is infected with both species. In these areas the epidemiological picture is confused because the developmental stages of *B. pahangi* and *B. malayi* are morphologically similar and definitive identification rests on structural differences in the spicules of the adult males, which can only be collected by biopsy or at autopsy. The criteria used for the identification of microfilariae in past studies were based on subtle differences in morphology which have proved unreliable. For example, Laing *et al.* (1960) said that *B. malayi* is shorter than *B. pahangi*, but Schacher (1962a) found that the length of microfilariae was too variable to be used to separate these species.

Although body length is unreliable, other microfilarial characters may be valuable in differential diagnosis such as the length of the Innenkörper (Sivanandam and Fredericks, 1966) and the length of the cephalic space or its length : width ratio (Schacher, 1962a). Recently the microfilariae of *B. pahangi* and subperiodic *B. malayi* have been separated by the distinct distribution of acid phosphatase activity on their surfaces (Redington *et al.*, 1975). Activity in *B. pahangi* is spread over the entire surface of the microfilaria while in *B. malayi* it is limited to the excretory and anal pores, the amphids and the phasmids. None of these methods has been confirmed or used on a routine basis in the field.

The mosquito stages of *B. pahangi* and *B. malayi* are also difficult to separate. Beckett and Macdonald (1971b) found that the rectal plug of *B. malayi* second-stage larvae from mosquitoes formed "a larger protuberance and was situated closer to the posterior end of the larvae, than was the case in *B. pahangi*". The infective larvae of *B. malayi* and *B. pahangi* cannot be differentiated on morphological grounds (Nelson, 1959; Schacher, 1962a). One wonders if the mosquito stages of *Brugia* spp. can be separated by the distribution of acid phosphatase activity by the methods of Redington *et al.* (1975).

Difficulties in the differential diagnosis of *Brugia* spp. in man has led to the development of xenodiagnostic tests. Wharton (1957a,b) observed variations in the development of *B. malayi* and *B. pahangi* in different species of mosquitoes and applied these differences to diagnosis. In *M. dives/bonneae* 44–100% of the subperiodic *B. malayi* larvae will develop to the third stage in 11 days while less than 6% of *B. pahangi* will complete their development. Small groups of mosquitoes were fed on 36 microfilarial carriers in East Pahang, West Malaysia. Since 48–100% of the larvae developed to the third stage in each group, it was concluded that all of the carriers were infected with *B. malayi* (Wharton, 1962). Although this conclusion is probably valid, this test does not eliminate the possibility that the carriers were also infected with *B. pahangi* because a proportion of the developing first- and second-stage larvae and up to 6% of the third-stage larvae could have been this species. To test for the presence of both filarial nematodes, a species of mosquito that is highly susceptible to

*B. pahangi* and relatively refractory to *B. malayi* such as *Armigeres subalbatus* (Wharton, 1962; Cheong *et al.*, 1965) or *Anopheles quadrimaculatus* (Orihel and Pacheco, 1966) should be used in addition to *M. dives/bonneae.*

The results of the above xenodiagnostic test can be confirmed by inoculating the infective larvae into susceptible vertebrates and examining the spicules of the adult males 6 weeks later. Once again, the validity of this test depends on the species of experimental vector as the results could be biased according to its differential susceptibility. *Brugia* infection has been passaged to animals from ten different carriers and in each case the parasite has been identified as *B. malayi* (Laing *et al.*, 1961; Guptavanij *et al.*, 1971b,c). The number of past attempts to infect animals was limited by the expense of maintaining carnivores and primates in the laboratory. The susceptibility of the jird to infection by *Brugia* spp. by the intraperitoneal route provides an inexpensive system for xenodiagnosis (Ash and Riley, 1970a,b; Ash, 1973b; McCall *et al.*, 1973).

### G. HYBRIDISATION OF *BRUGIA* SPP.

It might be argued that, with such minor morphological differences, *B. pahangi* and *B. malayi* are not true species. In fact, the last report of the World Health Organization Expert Committee on Filariasis stated: "It appears likely that the different forms of *B. malayi* are sibling species with *B. pahangi* in what is as yet an undefined complex" (WHO, 1974). To test this hypothesis, Denham *et al.* (1975) and Suswillo (unpublished) investigated the hybridisation potential of *B. pahangi*, *B. patei* and subperiodic *B. malayi.* Infected jirds were dissected when the worms had developed to the juvenile stage. Males or females were grouped and transplanted to separate jirds using a technique similar to that of Butts and Rabalais (1974). The worms were allowed to mature before being transferred to new jirds with members of the opposite sex from either their own or one of the other species. Microfilariae were produced in the peritoneal cavity in all combinations but one. The one infertile cross was between male *B. pahangi* and female *B. malayi* although the reciprocal cross was successful. Hybrid microfilariae were fed to mosquitoes using a membrane feeding technique (Ponnudurai *et al.*, 1971) and they grew to the infective stage. When these were inoculated into the peritoneal cavity of jirds, they developed to the fifth stage but the males failed to produce spermatozoa and microfilariae were not produced. It is clear that *B. patei*, *B. pahangi* and subperiodic *B. malayi* are biologic species.

### H. MAMMALIAN HOSTS OF SUBPERIODIC *BRUGIA MALAYI* AND *B. MALAYI*

There is a striking difference between the natural hosts of *B. pahangi* and *B. malayi. Brugia malayi* infects a number of primate hosts in nature whereas *B. pahangi* is found in a much more limited range of primate hosts (Laing *et al.*, 1960; Edeson, 1962). In carnivores the situation is reversed. *Brugia pahangi* more readily infects carnivores than does *B. malayi.* In West Malaysia, *B. pahangi* was found naturally infecting ten carnivores while *B. malayi* was limited to five (Table I). Even in these hosts *B. pahangi* was found in 20% of

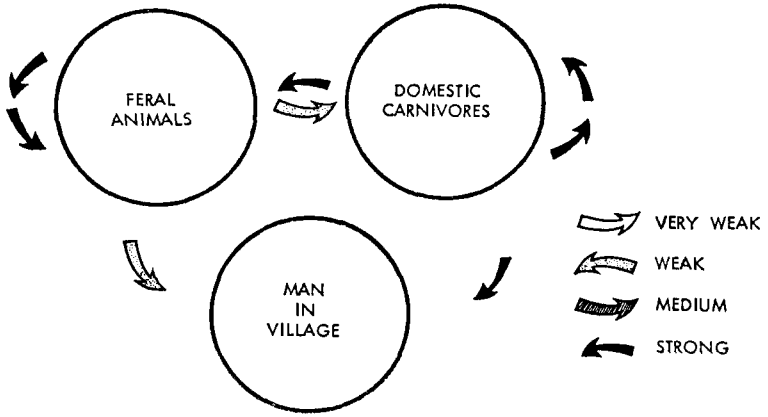


FIG. 5. The probable domestic and feral cycles of *B. pahangi*.

257 domestic cats and 44% of 32 civets while *B. malayi* was found in only 7% of the cats and 3% of the civets (Edeson, 1962). The probable relationship between *B. pahangi* and its domestic and feral cycles is presented in Fig. 5.

In the laboratory, cats are not as easily infected with *B. malayi* as with *B. pahangi*. Laing *et al.* (1961) found that 71% of 17 cats inoculated with periodic *B. malayi* became patent, but the levels of parasitaemia were low and several of the cats soon became amicrofilaraemic. The highest counts recorded were 0·58, 0·78 and 1·17 mf/mm$^3$ (microfilariae per cubic millimetre of blood) for three cats. Wilson and Ramachandran (1971) reported two other cats with peak counts of 0·67 and 1·58 mf/mm$^3$ and patency lasted at least 216 and 244 weeks, but they also state that "periodic *B. malayi* is much more difficult to transmit to animals", suggesting that there had been numerous failures.

Subperiodic *B. malayi* readily infected domestic cats in Kuala Lumpur. Edeson and Wharton (1958b) produced parasitaemias in all of 24 cats and Wilson and Ramachandran (1971) did not record a failure to produce patent infections in Malayan cats but said "the proportion of successful infections in English cats has been much lower". Whilst they do not publish evidence to support this claim, Burren (1972), who used the same parasitic strain, obtained microfilaraemias in only six of ten English cats and one of these was patent for only 3 weeks. Denham (unpublished) has confirmed these results.

*Brugia pahangi* readily infects both Malayan and English cats. Denham (1974a) found that 87% of 55 male cats and 90% of 59 female cats became patent. Infections with *B. pahangi* are usually long-lived (Wilson and Ramachandran, 1971; Denham *et al.*, 1972a) and microfilariae have been found up to 7 years after infection. There is no difference in the susceptibility of male and female cats (Denham, 1974a). Despite the ease with which English cats can be infected with the same strain of *B. pahangi*, there is still evidence that Malayan cats are more susceptible. Denham (1974a) reported that the

mean microfilarial levels in his cats for 10 weeks, starting three months after infection, were 4·27 mf/mm$^3$ for males and 2·52 mf/mm$^3$ for females. The peak counts for the cats used by Wilson and Ramachandran (1971) ranged from 17–189 mf/mm$^3$ with a mean of 21 mf/mm$^3$—some seven times higher than the figures obtained by Denham (1974a). The mean (peak) count for the subperiodic *B. malayi* infected cats of Wilson and Ramachandran (1971) was 4·9 mf/mm$^3$.

Laboratory infections of primates follow the pattern seen with natural infections. For example, *B. malayi* produced long-lasting patent infections in 88% of 34 *Macaca mulatta* (Fredericks and Ramachandran, 1969; El Bihari and Ewert, 1971) while *B. pahangi* produced transient microfilariae in 70% of ten *M. mulatta* (Denham, 1974b). In three monkeys, microfilariae were detected for 73, 50 and 35 days, but in the remaining animals microfilariae were either never seen or found on one or two occasions. Microfilariae of *B. malayi* circulate for a long time in *M. mulatta*—three monkeys showed microfilaraemia for 208, 112 and 76 weeks (Wilson and Ramachandran, 1971). *Macaca irus* is not as easily infected with *B. malayi*; Edeson and Wharton (1958b) found microfilariae in only 18% of 11 monkeys inoculated with *B. malayi*.

## I. DIAGNOSTIC TECHNIQUES

We agree with the WHO Expert Committee on Filariasis (WHO, 1974) that the parasitological demonstration of infection is the ideal way of diagnosing filariasis. The alternative to parasitological diagnosis is serodiagnosis. The advantage of serodiagnosis over conventional techniques is the ability to detect prepatent and postpatent infections as well as patent infection. The ideal serologic test should also provide information on the immune status of the patient in terms of reinfection and the development of disease. There has been a considerable effort devoted to the development of serological techniques, but the methods now available lack the required sensitivity and specificity to fulfil the above criteria (Ambroise-Thomas and Kien Truong, 1974).

In the past few years new techniques for the detection of microfilariae have been developed which are much more sensitive than the conventional stained smear. These include the modified stained smear, the counting chamber and the membrane filtration techniques. These techniques have been described in detail by Denham (1975) and the following discussion will only consider the advantages and disadvantages of each method.

The counting chamber method is the easiest technique for routine blood examinations. It is cheap, is accurately used after minimal training and, most important, utilises finger-prick blood. The maximum amount of blood that can be drawn from a finger-prick should always be used. This volume is certainly more than the 20–60 mm$^3$ samples which are so typical of past surveys. In a recent survey in East Africa 100 mm$^3$ was taken in routine examinations and in special circumstances the volume has been increased to 200 mm$^3$ or more.

In the original description of the counting chamber technique the micro-

filariae were counted immediately after lysing the blood cells with water (Denham *et al.*, 1971). It is not always convenient to examine blood samples immediately after they are taken, especially during night surveys. In this situation, up to 200 $mm^3$ of blood can be placed in 0·5 ml of 3% acetic acid which lyses the blood cells and preserves the microfilariae. These samples can be examined when convenient. They can also be stored for long periods of time (Wijers, pers. comm.).

The major deficiency with the counting chamber technique is the inability to make differential diagnoses and its use is limited in areas where two or more human filariids co-exist. In this situation, one must resort to the time-consuming stained smear technique. Denham *et al.* (1971) found that microfilariae drop off slides during processing and that thick film counts were 30–40% lower than chamber counts. Recently, Abaru and Denham (unpublished) used a modified thick film technique and found that their counts equalled those from the counting chamber. The modification simply involves mixing the blood sample with water to lyse the blood cells and allow the microfilariae to affix firmly to the slide. The smear is allowed to dry overnight, dehaemaglobinised by dipping it briefly in water and fixed and stained in the usual manner. In the conventional technique whole blood is allowed to dry on a slide, and it is likely that intact blood cells become sandwiched between a proportion of the microfilariae and the slide. These microfilariae might drop off the slide during the dehaemoglobinisation procedure when the sandwiched cells are lysed. It would be interesting to see if this simple modification of the thick smear technique would improve the detectability of other blood parasites.

The most sensitive method to detect microfilariae is the membrane filter technique. Southgate (1974) examined 366 people for Bancroftian microfilariae by four different techniques and found 22% of them positive on 20 $mm^3$ conventional blood smears, 30% positive on 60 $mm^3$ conventional smears, 39% positive in 60 $mm^3$ counting chambers and 68% positive by membrane filtration of 1 ml of blood. The membrane filtration technique is remarkably sensitive and is most useful in detecting patients with ultralow densities of microfilariae which often follow treatment with DEC. In theory, the sensitivity of the filter technique is limited only by the amount of blood which can be taken. One can pump up to 50 ml of blood through a single Nuclepore filter while 1–2 ml can be pumped through a single Millipore filter. Unfortunately, the filter technique has the disadvantages of being expensive and requiring personnel who are skilled at venepuncture. More important is the reluctance of people to subject themselves to venepuncture, and in many areas one can only hope to sample a small proportion of the population.

Since these techniques are applied to detect periodic microfilariae, it is important to consider the optimal time of day to collect blood samples. It is always best to take blood late at night when the number of microfilariae in the peripheral blood is highest. However, night disturbances often breed discontent in the population and it may be best to use the DEC provocative test in the daytime. With this test, blood is collected 30–60 min after the administration of 100 mg DEC. Partono *et al.* (1972) found no obvious differences in the infection rates between night smears and provocative day smears in areas

endemic for the nocturnally periodic strains of *W. bancrofti* and *B. malayi* and subperiodic *B. malayi*. Minor side-effects to the drug were observed in the *Brugia* areas, but these were not considered important.

## III. Vector–Parasite Relationships

All filarial nematodes use arthropods as intermediate hosts and literature reviews describing the various host–parasite combinations can be found elsewhere (Lavoipierre, 1958; Hawking and Worms, 1961; Nelson, 1964). During the past 12 years many advances have been made in this field and this information deserves a review of its own. In our discussion we include only studies which have more obvious practical application in laboratory maintenance of filarial nematodes for chemotherapeutic and immunologic studies or in the formulation of strategy to control filariasis in the field. Stress is given to adaptations of the parasite which facilitate its extrinsic development and transmission and to adaptations of the vector which serve as defence mechanisms to block filarial infection.

The sheathed microfilariae (first-stage larvae) of *Brugia* spp. circulate in the blood of their vertebrate hosts and are ingested by feeding mosquitoes. They are carried with the bloodmeal through the foregut to the midgut where they cast their sheaths and penetrate the stomach wall to the haemocoel. After migrating to the thorax they invade the muscle cells and develop to the third (infective) stage. Infective larvae leave the muscles and distribute themselves throughout the body cavities of the mosquito with a preference for the mouthparts. Transmission of these larvae from the mouthparts to the vertebrate occurs when infective mosquitoes take another bloodmeal.

### A. Ingestion of Microfilariae

There is much variation in the number of microfilariae ingested by individual mosquitoes fed at the same time on the same host. These variations may result from the distribution of microfilariae in the vascular system and from the feeding habits of mosquitoes. Gordon and Lumsden (1939) made direct observations on feeding mosquitoes and found that most of them took blood directly from the capillaries while a few ingested interstitial fluid. They also found that the number of microfilariae varied from capillary to capillary and that these differences were reflected in the number of worms ingested by mosquitoes. Experimental confirmation of these observations was provided by Kartman (1953), who found that the number of microfilariae ingested by mosquitoes fed on dogs infected with *Dirofilaria immitis* varied much more than the number taken by mosquitoes fed through a membrane on microfilariae "evenly" suspended in blood.

Kartman (1953) also noted that the number of *Dirofilaria* ingested by individual mosquitoes fed on dogs became more variable as the density of microfilariae in the circulation increased. This observation was extended to Bancroftian filariasis by Bryan and Southgate (1976), who found remarkably little variation in microfilarial ingestion when the donor presented an ultra-

low parasitaemia. They fed *Aedes polynesiensis* on a patient with 6 mf/ml of venous blood. In five experiments the infection rate varied only from 7–11% and the intensity of infection from 1–1·4 larvae per mosquito. These minimal variations make such experiments statistically satisfactory.

By using large groups of mosquitoes one can overcome the problem of variation in individual mosquitoes and determine if they ingest more or fewer microfilariae than calculated from the volume of blood which they imbibe. However, the results of these tests have been most confusing. The mean number of microfilariae ingested by mosquitoes was either more, less or equal to the expected value depending on the investigation (Gubler *et al.*, 1973; Bryan and Southgate, 1976). These contradictory results may be related to the techniques used to determine the density of microfilariae in the donor's blood as one can never be sure that samples from capillaries severed with a lancet or a vein punctured with a needle contain the same concentration of microfilariae as the blood from a capillary cannulated by the fascicle of a mosquito. Since the density of microfilariae in individual capillaries cannot be measured accurately, calculation of the number of microfilariae which a mosquito might be expected to ingest is likely to be erroneous.

The estimation of microfilarial intake has practical value in monitoring a control programme. It is particularly important in evaluating the impact of mass chemotherapy with DEC where control is achieved by reducing man's infectivity to mosquitoes. As part of the surveillance operation, the number of microfilariae ingested by vector mosquitoes should be calibrated against the density of parasites below which transmission ceases. Mass treatment should not be considered adequate until the parasitaemias of all carriers are below this critical level.

### B. REFRACTORY MECHANISMS IN THE FOREGUT

Immediately after ingestion, microfilariae encounter the cibarial and pharyngeal armatures. These structures are associated with the cibarial and pharyngeal pumps within the head capsule of the mosquito (Coluzzi and Trabucchi, 1968; Bryan *et al.*, 1974). The cibarial armature is a set of teeth which project into the posterior lumen of the cibarium while the pharyngeal armature is a set of sharp spines which project into the posterior lumen of the pharyngeal pump. Both armatures may mechanically abrade the sheaths and cuticles of microfilariae as they pass through the foregut, but the cibarial armature is the most lethal. The degree of damage depends on the size of the microfilariae and the size and structure of the armatures. The armatures of *Anopheles gambiae* species A and B and *An. farauti* kill around 90% of ingested microfilariae of *Brugia pahangi* (McGreevy *et al.*, unpublished). *Aedes aegypti* and *Ae. togoi* have only pharyngeal armatures and kill only 10%. Laurence and Pester (1961) found that 95–99% of *B. patei* also die in the stomach of *An. gambiae* and it is likely that they were killed by the armatures.

McGreevy *et al.* (unpublished) found that *An. gambiae* species A kills about 50% of the ingested *Wuchereria bancrofti* microfilariae while *Culex pipiens fatigans* and *Ae. aegypti* kill only 5%. The variation in the lethality of *An. gambiae* species A to *W. bancrofti* (45%) and *B. pahangi* (90%) is interesting

because the sizes of the microfilariae are similar. Although a West African strain of *An. gambiae* was used to study *B. pahangi* and an East African strain to study *W. bancrofti*, structural differences between their armatures were not found.

The lethal effects of the cibarial armatures on microfilariae are pertinent to programmes to control filariasis by mass chemotherapy with DEC. *Brugia malayi* and *W. bancrofti* microfilariae continue to circulate in low densities after DEC treatment (Desowitz and Southgate, 1973; Sajidiman *et al.*, 1975) and a small number of them are ingested by mosquitoes (Bryan and Southgate, 1976). The filaricidal effects of DEC and the cibarial armatures of the *Wuchereria* vectors should be cumulative in breaking transmission. None of the *Brugia* vectors have cibarial armatures and the effect of the pharyngeal armatures of the natural vectors on *Brugia* microfilariae is not known.

### C. REFRACTORY MECHANISMS IN THE MIDGUT

Shortly after feeding, the blood in the midgut of the mosquito coagulates. The speed of clotting varies between species and within species. Microfilariae may become trapped in blood which coagulates quickly and be prevented from penetrating the wall of the midgut. Since most microfilariae leave the stomach in the first 3 h, they are not trapped in the gut of slow coagulating mosquitoes. When mosquitoes were fed on a dog infected with *D. immitis*, 95% of the microfilariae left the gut of *An. quadrimaculatus*, a slow coagulator, while only 30% left the gut of *Ae. aegypti*, a fast coagulator (Kartman, 1953). However, when *Ae. aegypti* was fed through an artificial membrane on infected blood containing anticoagulants, 80% of the microfilariae left the gut. These results have been confirmed by Ewert (1965) using *B. pahangi* and *Ae. aegypti*. It can be concluded that blood coagulation is a major refractory mechanism that operates, at least, in *Ae. aegypti*.

These observations were extended by Obiamiwe and Macdonald (1973), who studied the susceptibility of *C. pipiens* to *B. pahangi*. In a susceptible stock, 21% of the microfilariae migrated to the haemocoel while in a refractory stock only 1% migrated. Parasite migration rates in the two stocks were equal when the mosquitoes were fed on blood containing anticoagulants. A series of crosses and backcrosses between these stocks were made and it was found that filarial development was controlled by a pair of sex-linked alleles with refractoriness dominant to susceptibility. Presumably, these alleles affect microfilariae indirectly by controlling the rate of blood coagulation. These alleles did not control the development of *W. bancrofti* (Macdonald *et al.*, 1973).

There is meagre circumstantial evidence that antifilarial toxins and/or digestive enzymes operate in the midgut of *Culex* mosquitoes, but not in *Aedes*. Kartman (1953) found that the microfilariae of *D. immitis* survive in the blood clots of *Ae. aegypti* and *Ae. albopictus* more than 72 h, but live in *Culex quinquefasciatus* (=*C. p. fatigans*) and *C. pipiens* for only 12–24 h. If such antifilarial substances exist in *Culex* spp. they are probably unimportant because they kill only those worms which were already trapped in the blood clot and are unlikely to migrate to the haemocoel.

It has also been suggested that the peritrophic membrane of mosquitoes limits filarial migration, but the evidence is only circumstantial. Histological studies on the gut indicate that most worms migrate from the stomach prior to formation of the peritrophic membrane and that migration ceases about the time the membrane becomes well developed (Esslinger, 1962; Bain and Brengues, 1972). Although this may be so, these studies do not account for other changes in the gut which occur at the same time such as coagulation of blood. Experimental evidence is needed to determine if microfilariae can or cannot penetrate the well-developed peritrophic membrane of mosquitoes.

The relationship between the number of microfilariae ingested and the proportion of those which successfully migrate to the haemocoel has been determined in four filaria–vector systems (Bain, 1971; Brengues and Bain, 1972; Bain and Chabaud, 1975). In one system, *W. bancrofti–An. gambiae* species A, the proportion of microfilariae that migrated to the haemocoel increased as the number of ingested parasites increased, a phenomenon they referred to as "facilitation". In the other systems, *W. bancrofti, Setaria labiato-papillosa* or *Dipetalonema dessetae* in *Ae. aegypti*, they found that the proportion of microfilariae which migrated to the haemocoel decreased as the number of microfilariae ingested increased. This was called "limitation".

Histological studies on the penetration of the gut by microfilariae have been made by Bain and Philippon (1970), Bain and Brengues (1972) and Bain and Chabaud (1975). They found intracellular microfilariae in the columnar-shaped cells of the anterior and posterior portions of the midgut. Since microfilariae were not seen in the stretched "squamous"-shaped cells surrounding the remainder of the bloodmeal, the authors suppose that penetration occurs only through columnar cells. In *An. gambiae* the parasitised cells were disorganised with basophilic cytoplasms and pycnotic nuclei and the surrounding epithelium was hypertrophic. Bain and Brengues (1972) and Bain and Chabaud (1975) speculate that the creation of hypertrophied areas in reaction to the first penetrating microfilariae increases the number of sites where subsequent microfilariae can penetrate and, therefore, facilitates their migration to the haemocoel. In *Ae. aegypti* there was no hypertrophy, and damage was usually limited to the one or two cells that had been attacked by microfilariae. The nuclei were pycnotic and the cytoplasm basophilic. Bain and Chabaud (1975) believe that this reaction somehow impedes the penetration of the midgut epithelium by microfilariae. Although these speculations may be true, it seems more likely that facilitation and limitation in the various filaria–vector combinations is the summation of microfilarial interactions with the pharyngeal and cibarial armatures, the blood clot, digestive enzymes, putative anti-filarial toxins, peritrophic membrane and the gut epithelium.

When *An. gambiae* feeds on Bancroftian carriers with ultralow densities of microfilariae (1–60 mf/ml), most of them fail to ingest any microfilariae while the others take only one or rarely two (McGreevy *et al.*, unpublished). Brengues and Bain (1972) show that about 80% of these microfilariae would fail to migrate to the haemocoel; 45% being killed by the armature (McGreevy, unpublished) and 35% being killed by other causes. When *Ae. aegypti* ingests small numbers of microfilariae, 60% of them migrate to the haemocoel; only

5% are killed by the armature and 35% are killed by other causes. These results indicate once again that the control of filariasis with DEC is likely to be most effective in areas where the vectors are more efficient in limiting development of the parasite.

### D. REFRACTORY MECHANISMS IN THE THORACIC MUSCLES

Even though microfilariae may evade all of the refractory mechanisms that operate in the gut and successfully invade the cells of the thoracic muscles, their development is not guaranteed. In some strains of *Ae. aegypti* both *Brugia* and *Wuchereria* develop to the third stage while in other strains the infection is aborted soon after penetration of the muscles (Ewert, 1965; Laurence, 1970). Reciprocal crosses between individuals of these two strains showed that susceptibility to filarial infection is an inherited character controlled by simple sex-linked alleles with refractoriness being dominant to susceptibility (reviewed by Macdonald, 1976). The genes controlling the development of *Brugia* spp. and *W. bancrofti* in the thoracic muscles are not identical to those controlling *D. immitis* and *D. repens* in the malpighian tubules (Macdonald and Ramachandran, 1965; Zielke, 1973; McGreevy *et al.*, 1974a; Coluzzi and Cancrini, 1974). It is likely that separate genes directly affect the developmental tissue, which in turn affects the filarial larvae. Genes affecting the thoracic muscles are called *fm* (filarial susceptibility, *B. malayi*) and those affecting the malpighian tubules are called *ft* (filarial susceptibility, malpighian tubules).

The genetic basis for the susceptibility of the *Aedes scutellaris* complex to infection with *B. pahangi* is currently under investigation (Macdonald, 1976). Reciprocal crosses between susceptible *Ae. polynesiensis* and refractory *Ae. malayensis* produce refractory hybrid females suggesting that refractoriness is dominant to susceptibility. Backcrosses to parental *Ae. malayensis* produce refractory progeny. However, the more informative backcross to *Ae. polynesiensis* has not been made and conclusions on mode of inheritance await these results. Since *Ae. malayensis* is refractory to both *B. pahangi* and *W. bancrofti* and *Ae. polynesiensis* is susceptible to both species, it is hoped that the same putative gene will control the development of both parasites.

Information on the method of expression of refractory genes is meagre. Townson (1974) infected mosquitoes by injecting exsheathed microfilariae of *B. pahangi* into their thoraces. The larvae developed to the third stage equally well in both male and female *Ae. aegypti* from susceptible stocks, but did not develop in females from refractory stocks. However, complete larval development occurred in 40% of the male *Ae. aegypti* taken from the refractory stock. Similar observations were made in *Ae. malayensis* (Townson, 1975). Preliminary investigations on the heterogeneity of males of refractory stocks indicate that susceptibility is a heritable character.

The development of larvae in male mosquitoes suggests that filarial growth and differentiation is independent of female hormones and that susceptibility is not related to the gonadotrophic cycle. This suggestion is supported by Gwadz and Spielman (1974), who found that the development of *B. pahangi* in susceptible females of *Ae. aegypti* and the lack of development in refractory

females was not altered by extirpation of the corpora allata or median neurosecretory cells or exposure to synthetic analogues of juvenile hormone or ecdysterone.

It also appears that the expression of refractory genes in *Ae. aegypti* is not related to the immune response because the dead *Brugia* larvae found in the thorax are rarely melanised or encapsulated (Beckett and Macdonald, 1971a). However, there is circumstantial evidence suggesting that melanisation may kill developing *Brugia* in other mosquito species (Oothuman *et al.*, 1974).

The refractory genes discussed above appear to stop the growth and differentiation of first stage larvae in the thoracic muscles of *Aedes* species. However, even in susceptible mosquitoes (i.e. *fm*/*fm*) a large proportion of the larvae die in the thorax; 25% of the first stage larvae of *B. pahangi* and 75% of the larvae of *B. malayi* die within the first 5 days (Beckett and Macdonald, 1971a). This phenomenon may indicate that susceptibility in the thoracic muscles is controlled by other factors in addition to the *fm* alleles.

### E. ORIGIN OF REFRACTORY MECHANISMS

The refractory mechanisms of mosquitoes may have evolved in response to unknown selection pressures, but it is tempting to speculate that they arose largely in response to filarial infection. Filarial nematodes are pathogenic to mosquitoes and may reduce their longevity and biotic potential depending on the intensity of infection. Townson (1971) infected homozygous susceptible, heterozygous and homozygous refractory *Ae. aegypti* with *B. pahangi* and confirmed earlier reports that the survival of refractory individuals is significantly higher than that of susceptible individuals. Using the same system, Javadian and Macdonald (1974) studied the fecundity of the infected survivors and found that refractory mosquitoes laid more eggs than the susceptible ones. In both studies the effects on survival and fecundity were inversely related to the intensity of infection.

Using the hypothesis that filarial infection selects against susceptible mosquitoes, Macdonald (1964, 1965) attempted to change the gene frequency of a heterologous population *Ae. aegypti* by feeding them on cats infected with *B. malayi*. However, after 14 mosquito generations, the proportions of susceptible and refractory individuals in the experimental cages was similar to those in control groups that were fed on normal cats. Although these results appear to contrast with those of Townson (1971) and Javadian and Macdonald (1974), it is possible that Macdonald (1964, 1965) fed his mosquitoes on cats with such low densities of microfilariae that pathogenic infections were not produced. This experiment should be repeated before it is accepted that filarial infection does not select for refractoriness.

Analysis of the frequency of *fm* in several populations of *Ae. aegypti* in East Africa suggests that *W. bancrofti* may select for refractoriness in nature. Rodriguez and Craig (1973) showed that domestic populations of *Aedes* tended to be refractory compared to sylvatic populations which rarely bite man. *Wuchereria* microfilariae are often ingested by domestic *Aedes* in East Africa and it is possible that *W. bancrofti* infection selected for refractory *Ae. aegypti*. How-

ever, it is also possible that refractoriness could have been selected by animal filariae which infect the thoracic muscles of mosquitoes.

### F. CO-EVOLUTION OF FILARIA–MOSQUITO SYSTEMS

In the face of vector refractoriness there must be selection pressure on the parasite to evolve counter-measures. It is pertinent to note that refractory mechanisms never seem to be completely effective and small numbers of filarial larvae often evade the mosquito defence system to develop to the third stage. Even the most lethal pharyngeal and cibarial armatures fail to kill all of the ingested microfilariae. The coagulation of blood does not prevent all of the larvae from migrating to the haemocoel, and refractory genes do not arrest the growth and development of every larva in the developmental site. To explain the failure of homozygous refractory *Ae. aegypti* to arrest all *Brugia* larvae in the thorax, Macdonald (1963) proposed that the dominant genotype was incompletely expressed. Although penetrance may be involved in relative refractoriness, it is odd that such a failure in expression in an individual mosquito and even in an individual muscle fibre may arrest some larvae and have no effect on others which proceed to develop to the third stage (Beckett and Macdonald, 1971a). These observations indicate that both mosquito refractoriness and filarial infectivity vary from one individual to the next. The outcome of a mosquito–filarial worm relationship depends not only on the relative refractoriness of the mosquito, but on the relative infectivity of the ingested microfilariae. Such a phenomenon may account for the acquisition of new vector species by filarial nematodes as it is probable that the most infective filarial larvae are able to develop in the least refractory vectors (McGreevy *et al.*, 1974a).

Support for this hypothesis was provided by Laurence and Pester (1967), who altered the infectivity of *B. patei* to *Ae. togoi*. Initially, only 44% of the mosquitoes became infected and the surviving third-stage larvae were passaged to cats. After six generations the parasite was able to infect 90% of the mosquitoes. They demonstrated that the microfilariae circulating within a host constitute a population composed of mutants and genetic recombinants that vary in infectivity for their vector.

There has been considerable discussion on the control of filariasis by introducing refractory genes into natural populations (Macdonald, 1976). The results of Laurence and Pester (1967) indicate that the target filarial worm will simply adapt to the new vector strain.

## IV. DEFINITIVE HOST–PARASITE RELATIONSHIPS

### A. TRANSMISSION OF INFECTIVE LARVAE

Although a few infective larvae escape from mosquitoes feeding on sugar, raisins or other sources of carbohydrate (Bemrick and Bemrick, 1969), the vast majority leave the mouthparts of mosquitoes while they are biting vertebrates. The most important stimuli for larval escape are associated with the act of blood feeding (Lavoipierre and Ho, 1966, 1973; Ho and Ewert, 1967; McGreevy

*et al.*, 1974b). The actual ingestion of blood and warmth from the skin are of little importance because larvae escape while mosquitoes probe the skin before feeding and from mosquitoes probing the skin of dead mice. The major stimulus for larval escape is the bending of the labium; most larvae escape while the labium is acutely bent; few, if any, escape while the labium is straight.

Considerable attention has been given to the exact site from which infective filarial larvae escape. McGreevy *et al.* (1974b) observed that larvae of *Dirofilaria immitis* most frequently escape from the tips of the labellae directly on to the skin and rarely from the mid-portion of the labium. Larvae which escape from the mid-portion of the labium are less likely to reach the skin as they must travel to the tip of the proboscis while the mosquito is feeding or be carried away on the engorged female.

Having reached the surface of the skin, infective larvae enter the puncture wound which remains after withdrawal of the mosquito fascicle. Infective larvae cannot invade intact skin and depend on the puncture wound for infection. Ewert (1967) and Ewert and Ho (1967) made direct observations on the transfer of *Brugia* larvae from infective *Aedes togoi* to the skin of mice. Larvae were found in tissue sections of skin if the infective mosquitoes were allowed to feed to repletion and withdraw their fascicles, but were not seen in tissue sections if the fascicle was severed before it could be withdrawn from the skin. Although larvae emerged from these mosquitoes the puncture wound was blocked by the fascicle which remained *in situ* and no larvae were found in the tissues of the mice.

Survival of larvae on the surface of the skin is a crucial event. When the infective larvae escape from the labellae a small drop of insect haemolymph oozes on to the surface of the skin (Ewert, 1967; Ewert and Ho, 1967; McGreevy *et al.*, 1974b), which protects the larvae from desiccation and provides them with a medium in which to find the puncture wound of the mosquito. Under laboratory conditions this haemolymph desiccates within 4 min and larvae that have failed to penetrate the skin during this period die. Analysis of the data of Ho and Ewert (1967) and Ewert and Ho (1967) show that 62–90% of *B. pahangi* larvae that emerge from *Ae. togoi* on to the surface of the skin die from desiccation. The proportion of larvae which die on the surface of the skin is directly related to the rate of desiccation of the haemolymph drop (McGreevy *et al.*, 1974b). Rapid desiccation reduces the time available for infective larvae to invade the puncture wound and leaves the less fortunate individuals to die on the skin. Since the rate of evaporation of the haemolymph drop is dependent on the relative humidity, the longevity of this fluid and the success of transmission should increase as the humidity of the microclimate approaches saturation. It is interesting that human filariasis is limited to the more humid areas of the tropics.

We have been investigating the link between humidity and transmission. *Aedes aegypti* infected with *B. pahangi* have been allowed to feed on the feet of cats at 80% RH or at 20% RH. The number of infective larvae that emerged from the proboscis of the vector was estimated by dissecting groups of mosquitoes before and after feeding. After feeding, the number of larvae that died on the surface of the skin was determined by washing the feet. One day later

the cats were dissected to determine the number of larvae that penetrated the skin and migrated to the regional lymph node. The results of this experiment were a surprise as 90% of the larvae deposited on the skin of the cats under conditions of high or low humidity invaded the skin. However, in the dry environment, very few larvae escaped from the mosquitoes relative to the numbers which escaped in the humid environment (Suswillo *et al.*, unpublished).

### B. THE DEVELOPMENTAL CYCLE IN THE MAMMALIAN HOST

In primates and carnivores, development of *Brugia* spp. occurs in the lymphatic system. In most experimental studies larvae have been inoculated subcutaneously, although Ewert and El Bihari (1971) used a technique whereby

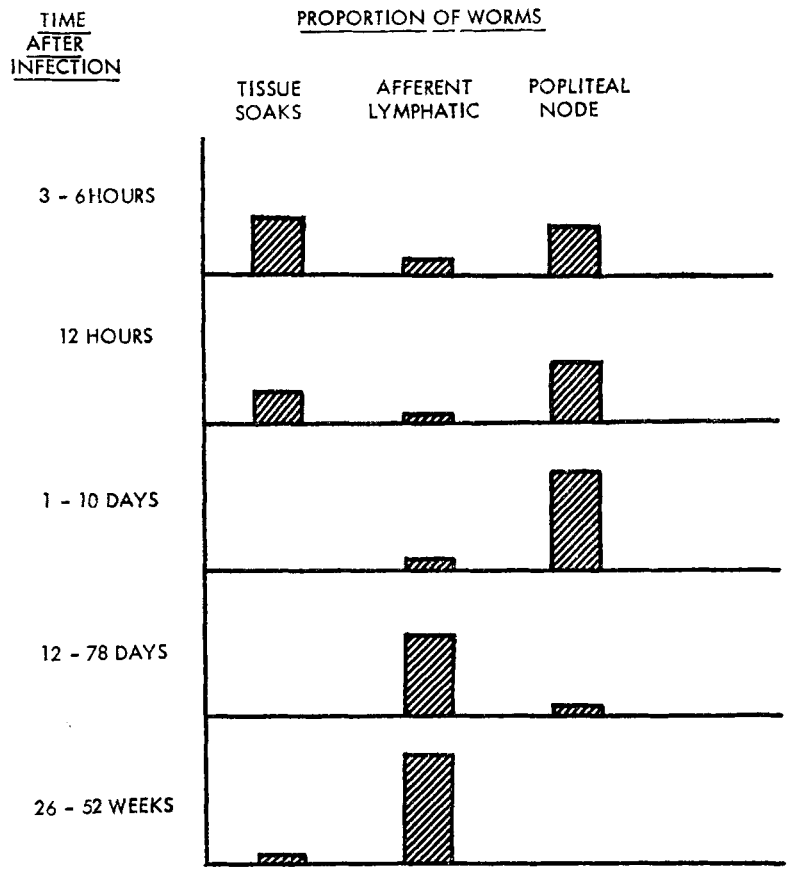


FIG. 6. Migration of *B. pahangi* after subcutaneous injection of infective larvae into the hind feet of cats (Suswillo *et al.*, unpublished).

the skin was repeatedly pricked with a pin and larvae applied to the injured area. They showed that larvae penetrated the puncture wounds in a manner similar to that occurring during mosquito transmission. Animals can also be infected orally (Gwadz and Chernin, 1973) or by ocular infusion (Ah *et al.*, 1974b). Whether infection is by mosquito bite, inoculation, or the pin-prick method, the end result is the same. In cats and monkeys the larvae penetrate the local lymphatics within a few hours of infection and migrate with the flow of lymph to the perinodal lymphatic sinus of the nearest lymph node (Ewert and El Bihari, 1971; Ewert and Ho, 1967; Suswillo *et al.*, unpublished). Later they migrate against the flow of lymph and return to the afferent lymphatic where they remain for the rest of their life. The time of migration of *B. pahangi* and *B. malayi* differs considerably (Figs 6, 7). Suswillo *et al.* (unpublished) found that during the first 12 h after inoculation of *B. pahangi* into the hind foot of cats, third-stage larvae were recovered by soaking the tissues of the lower leg in saline or by dissecting the popliteal lymph node; only a few larvae were found in the afferent lymphatic. Between 1 and 10 days after infection, most larvae were found in the lymph node with a few in the afferent lymphatic. From 12 days onwards, few larvae were in the lymph nodes and the large majority were in the afferent lymphatic. It is no coincidence that the first enlargement of the lymphatics is noted about 14 days after infection (Section VB). In contrast to *B. pahangi*, adult *B. malayi* are found in the perinodal sinus as late as 13 weeks after infection (Ewert, 1971) at a time when most adult *B. pahangi* are found only in the afferent lymphatic.

The morphological changes during maturation of the worms in the mammal

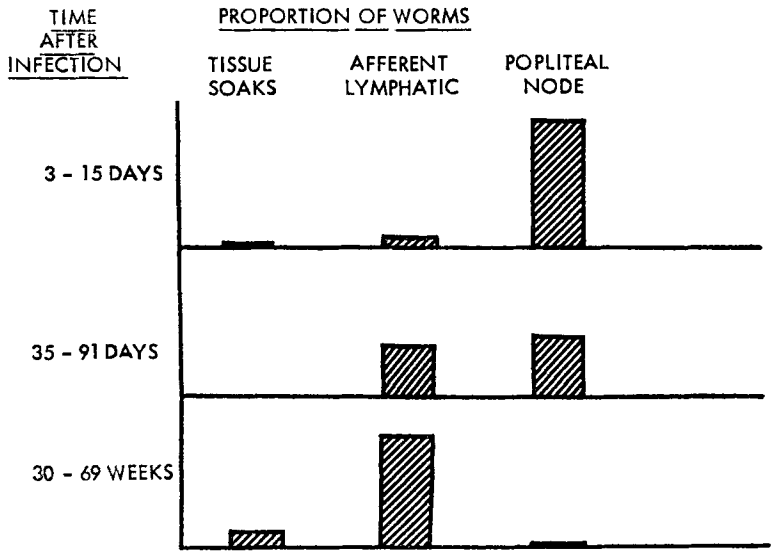


FIG. 7. Migration of *B. malayi* after their penetration of the punctured skin of the feet of cats (Ewert, 1971).

were described by Schacher (1962b). He worked with *B. pahangi* but the development of *B. malayi* and *B. patei* are very similar except for details of timing (Buckley, pers. comm.). Since the discovery that *B. pahangi*, *B. malayi* and *B. patei* will successfully develop in the peritoneal cavity of the jird (*Meriones unguiculatus*) (Ash and Riley, 1970a,b; Ash, 1973b) more accurate timing of the morphological changes has been possible although the rate of development in the jird may well be very different from that in the natural host. The first moult occurs about 9 days after infection and it is only after this moult, during the fourth larval stage, that growth commences. The final moult occurs around 25 days and varies between the species. The fifth-stage worm continues to grow. Microfilariae can be found in the blood of cats infected with *B. pahangi* on day 54 and this time also varies between the species.

### C. INFECTIONS OF RODENTS WITH *BRUGIA* SPP.

It is very expensive to use cats, dogs or monkeys as experimental hosts and there have been numerous attempts to introduce *Brugia* into commonly available laboratory rodents. The most successful attempts have been with the mongolian jird, *M. unguiculatus*, which is a host for *B. pahangi*, *B. malayi* and *B. patei* (Ash and Riley, 1970a,b; Ash, 1973a). Most of the original infections were initiated by inoculating infective larvae subcutaneously. In this type of infection adult worms are found in the heart, pulmonary arteries, lymphatics and testes (Ash and Riley, 1970a,b; Ash, 1971, 1973a; Ah and Thompson, 1973; El Bihari and Ewert, 1971). However, if the larvae are inoculated intraperitoneally they develop to the adult stage free in the peritoneal cavity with the exception of a few which penetrate the lymphatics of the spermatic cord or peritoneum (Ah and Thompson, 1973; McCall *et al.*, 1973).

Ash (1971) reported that male jirds were much more susceptible to infection than were females; 82% of 17 males and 24% of 17 females become patent after subcutaneous inoculation of 25–75 *B. pahangi* larvae. El Bihari and Ewert (1973) found a similar difference between the two sexes when they were infected with *B. malayi*: 96% of 26 males and 43% of 21 females became patent after infection with 20–105 larvae. Ash (1971) found a mean prepatent period of 72 days for male jirds and 80 days for female jirds infected with *B. pahangi* while El Bihari and Ewert (1973) found the mean for *B. malayi* was 129 days for males and 117 days for females. Because of the reliability of *Brugia* infections in the jird, this system is now being used in a variety of studies.

Although there are few reports on *Brugia* infections in the multimammate rat (*Praomys natalensis*) this system also has promise. Petranyi and Mieth (1974) found that 76% of 92 males became patent after inoculating 100 *B. malayi* infective larvae. Adult worms were found in the lungs (40%), lymphatics (36%) and testes (24%). Ahmed (1966) successfully infected six animals with *B. pahangi* and Benjamin and Soulsby (1974) used this system in an immunological study.

The golden hamster, *Mesocricetus auratus*, has also been infected with *Brugia* spp. and about 35% of them develop patent infections with *B. pahangi* and 20% become patent with *B. malayi* (Laing *et al.*, 1961; Edeson *et al.*, 1962;

Ash and Riley, 1970b; Sucharit and Macdonald, 1972; Harbut, 1973; Malone *et al.*, 1974). The adult worms have been recovered from the heart, pulmonary vessels, lymphatics and testes.

The most desirable laboratory hosts for *Brugia* are the rat and mouse. There is a wealth of information on these species and research is facilitated by the ready availability of inbred strains as well as a variety of biochemical and immunological reagents. Unfortunately, these animals are not as susceptible to *Brugia* as they might be. Only 19% of 26 rats became patent with *B. malayi* (Laing *et al.*, 1961; Cheong *et al.*, 1967) while 15% of 146 rats became patent for *B. pahangi* (Ash and Riley, 1970a; Sucharit and Macdonald, 1972; Harbut, 1973). There has been one attempt to select a "rat strain" of *B. pahangi* with little success; the patency rate was 12% of 101 rats in the parental generation and 17% of 127 in the F4 (Sucharit and Macdonald, 1973).

Attempts to infect mice have been even more discouraging. Laing *et al.* (1961) and Ahmed (1967) found no sign of infection in mice inoculated with infective larvae while Chong and Wong (1967) inoculated three strains of mice with *B. pahangi* with rather more success. Although the mice did not become patent, adult worms were recovered from the lymphatics and heart of two individuals.

Apart from the above experiments, there are a few attempts to infect a variety of other rodents. Striebel (pers. comm.) obtained patent infections with *B. malayi* in eight of 23 *Saccostomus campestris* while Ash and Riley (1970b) found only one microfilaria in one of three *Meriones libycus*. Attempts to infect the following rodents with *Brugia* have been unsuccessful: rabbits and guinea-pigs (Ahmed, 1967), *Gerbillus hirtipes* (Striebel, pers. comm.), *Dipodomys merriami* and *Neotoma lepida* (Ash and Riley, 1970a,b).

## V. Pathology

### A. The human disease

There have been many reports on the clinical effects of *Brugia malayi* infection in man (Lichtenstein, 1927; Turner, 1959a; Dondero and Menon, 1972). Lichtenstein (1927) was the first to report the differences in the diseases caused by *B. malayi* and *Wuchereria bancrofti*. He noticed that elephantiasis of the legs was very common in Northern Sumatra but the other sequelae of Bancroftian filariasis, such as elephantiasis of the scrotum, hydrocoel and chyluria were noticeably absent. These observations have been confirmed from other regions where *B. malayi* is endemic. Whilst most studies concern patients with the nocturnally periodic strain of *B. malayi*, there is little difference between clinical disease caused by the periodic and subperiodic strains (Turner, 1959a).

It has been generally reported that most people infected with *B. malayi* have swollen lymph nodes, but Edeson (1955) found as many enlarged lymph nodes in children from an area with no filariasis as in children from an area of endemic filariasis. Turner (1959a) found that only half the patients with Malayan filariasis had enlarged lymph nodes and that the femoral and superficial in-

guinal nodes were most commonly enlarged. Turner summed up this point neatly when he said, "The presence of enlarged lymph nodes does not indicate filariasis; their absence does not exclude it."

One of the temporarily disabling features of Malayan filariasis is the so-called "filarial fever". Turner coined the phrase "acute (episodic) adeno-lymphangitis" (AEAL) to describe this syndrome; AEAL starts, typically, with a painful and tender enlargement of a superficial inguinal lymph node with accompanying fever. Within a few hours a red streak develops over the lymphatics. This is usually localised to an area approximately $6 \times 1$ cm. The fever and other symptoms continue for 3–5 days and AEAL resolves in the next 6–7 days. There is usually lymphoedema of the foot on the affected side and this increases in severity and duration with each successive occurrence of AEAL. Only one site at a time is affected by AEAL, which almost always involves the lower limbs. Abscesses are commonly associated with the areas affected by AEAL (Dassanayake, 1938; Poynton and Hodgkin, 1938).

Elephantiasis, which is probably the most feared and unpleasant sequel to Brugian filariasis, is largely restricted to the legs. Taking all the figures produced by Turner (1959a) the overall prevalence of elephantiasis in different sites is 92 % in the legs, 2 % in the arms and 1 % in the scrotum (ignoring patients with multiple involvement).

Turner (1959a) gives a figurative representation of the course of events in human Brugian filariasis; he says that "the course of filariasis is variable and unpredictable". Although the timing of events for any individual may be variable, the sequence is almost invariable. From Turner's results, the following points emerge:

(1) Brugian filariasis with microfilaraemia is comparatively inocuous.
(2) The onset of AEAL is likely to lead to more severe attacks with the beginnings of lymphoedema of the lower limbs. As the attacks of AEAL become more severe, lymphoedema becomes more persistent.
(3) Eventually microfilariae disappear from the blood, lymphangitis becomes a more permanent feature of AEAL and overt elephantiasis replaces episodic lymphoedema.

Despite this simplified picture, it must be recognised that not all patients with elephantiasis are amicrofilariaemic nor do all patients whose blood becomes amicrofilariaemic develop elephantiasis.

## B. PATHOLOGICAL REACTIONS IN EXPERIMENTAL ANIMALS

### 1. *Gross reactions*

One would expect that such large worms as adult *Brugia* spp. in the lymphatics and lymph nodes would cause considerable damage. Although there are dramatic gross and histopathological changes in the lymphatics and lymph nodes, externally visible signs of infection are rare in experimental infections. Lymphoedema has been produced in cats and dogs infected with *B. pahangi* and *B. malayi* (Schacher *et al.*, 1969, 1973; Rogers and Denham, 1974), but in each case the lymphoedema has been transitory and has not progressed to

become elephantiasis as it would in man. Bosworth *et al.* (1973) produced dramatic gross changes, including extensive ulceration, in cats infected with *B. malayi* and feline streptococci.

Denham *et al.* (1972b) showed that a proportion of cats that are repeatedly infected over long periods suddenly become amicrofilaraemic. At the same time, they develop transient, and often recurring, lymphoedema (Rogers and Denham, 1974). At this time, the inoculation of more infective larvae causes the foot and lower limb to become enlarged. In some cats this phase is very transient and after a few weeks the inoculation of larvae does not cause swelling; in other cats, the swelling lasts for several weeks. The unpredictable nature of this reaction makes a detailed study very difficult.

After a single infection with any *Brugia* spp., cats never exhibit any sign that they are distressed by infection. They never object to having their lymph nodes and lymphatics palpated. Soon after infection, palpation of the infected limb shows that the popliteal lymph node is much larger than in the uninfected contralateral limb. From about 2 months after infection it is possible to palpate the swollen, varicosed lymphatic afferent to the popliteal lymph node in most cats.

The sequential damage to lymphatics parasitised by *Brugia* spp. may be studied either in living animals by lymphography or post-mortem. Post-mortem examination has the advantage that a full dissection followed by histological studies can be made but has the distinct disadvantage that with expensive animals so few can be used that one may be misled. Schacher and Sulahian (1972) combined such a study with a detailed analysis of lymphatic drainage patterns in dogs infected with *B. pahangi* and *B. malayi*. They used 15 dogs infected for 2–89 days with *B. malayi* and nine dogs infected for up to 4 years with *B. pahangi* and one dog which had received six inoculations of *B. malayi*. They infected both hind limbs of their dogs, which makes interpretation of some of their results difficult as lymph nodes vary in size from animal to animal. If both legs are infected, one cannot compare the normal limb with the infected limb. However, they made some very interesting observations on the lymphatics and noted that dilatation had begun by 21 days post-infection. As a result of these observations, they suggested: "the net damage to the lymphatic system is probably a product of worm-burden × time. A few worms acting over a long span may have about the same net effect as many worms acting for a shorter period." This is an interesting concept which, if it were true, would mean that damage would be progressive for as long as the animal remained infected. This is not so for *B. pahangi* infections of the cat. Rogers and Denham (1974) compared lymphangiograms performed on cats some months before autopsy with post-mortem observations. In no case was gross pathology more advanced at the time of death than it had been when the lymphangiograms were performed. Using the new technique of xeroradiography plus Hypaque (Rogers *et al.*, 1975a), Rogers and Denham (unpublished) have shown that in cats given a single inoculation of larvae into one foot, maximal damage occurred about 75 days after infection and this damage slowly regressed. The damage to the lymphatic system in repeatedly inoculated cats was progressive both in terms of the intensity of reaction in the lymphatics

initially infected and in the amount of lymphatic system affected (Rogers and Denham, 1974). In these cats, the lymph nodes were only a little bigger than those given a single infection, but the afferent lymphatics could usually be palpated and were hard and ropey. After a large number of repeated infections, diseased lymphatics could be seen underlying the skin, particularly on the inside of the thigh. Following hyperinfection of the normal afferent lymphatics by repeated inoculations, collateral lymphatics develop, which then become infected and diseased (Ewert and Bosworth, 1975). Rogers and Denham (unpublished) used the xeroradiographic technique to study repeatedly infected cats and confirmed the gradual extension of the affected area as the number of infections increased. *Brugia malayi* and *B. patei* infections are much more pathogenic in the cat than are *B. pahangi* infections. For example, one seldom sees the development of collateral lymphatics after a single infection with *B. pahangi* (Gooneratne *et al.*, 1971) whereas after a single infection with *B. malayi* such collateral lymphatics are common (Ewert *et al.*, 1972).

2. *Reactions in lymph nodes*

Two histologically distinct types of immunological responses occur in lymph nodes (Turk, 1969; Weiser *et al.*, 1969; Anderson and McKeating, 1970; Cottier *et al.*, 1972). The cell-mediated-type immunological (CMI) response is characterised by proliferation of lymphocytes in the paracortical region of the lymph node and the antibody-type (AB) response by proliferation of lymphocytes in germinal centres and production of large numbers of plasma cells in the cortico-medullary junction and medullary cords of the nodes.

Rogers *et al.* (1975b) related histological changes in the lymph nodes of cats infected with *B. pahangi* to those induced by agents known to initiate either a cell-mediated or an antibody-mediated response (Fig. 8). There was a very rapid onset of a CMI response which began to wane from 2 months after infection so that old infections had about the same degree of CMI activity as did "normal" cats. The AB response was much slower in starting and for about a week the nodes were barely different from those of normal cats or from the contralateral limb of the same cats. By 14 days there was a pronounced AB response in the nodes which continued at a high level for the 2 years during which observations were made.

Schacher and Sahyoun (1967) have given a detailed account of the other histopathological changes in lymph nodes of cats and dogs infected with *B. pahangi*. They considered that many of the changes were related to living and dead worms, moulting, and the production of uterine mucus, sterile ova and microfilariae. In contrast to these results, we have found that pathologic changes in cats are not specifically correlated with these parasitologic events.

3. *Reactions in lymphatics*

Rogers and Denham (1974) published a detailed account of the changes occurring in the lymphatics of cats infected with *B. pahangi*. Histological change occurred almost immediately after infection and long before any gross change could be seen. In the first week after infection there was slight lymphan-

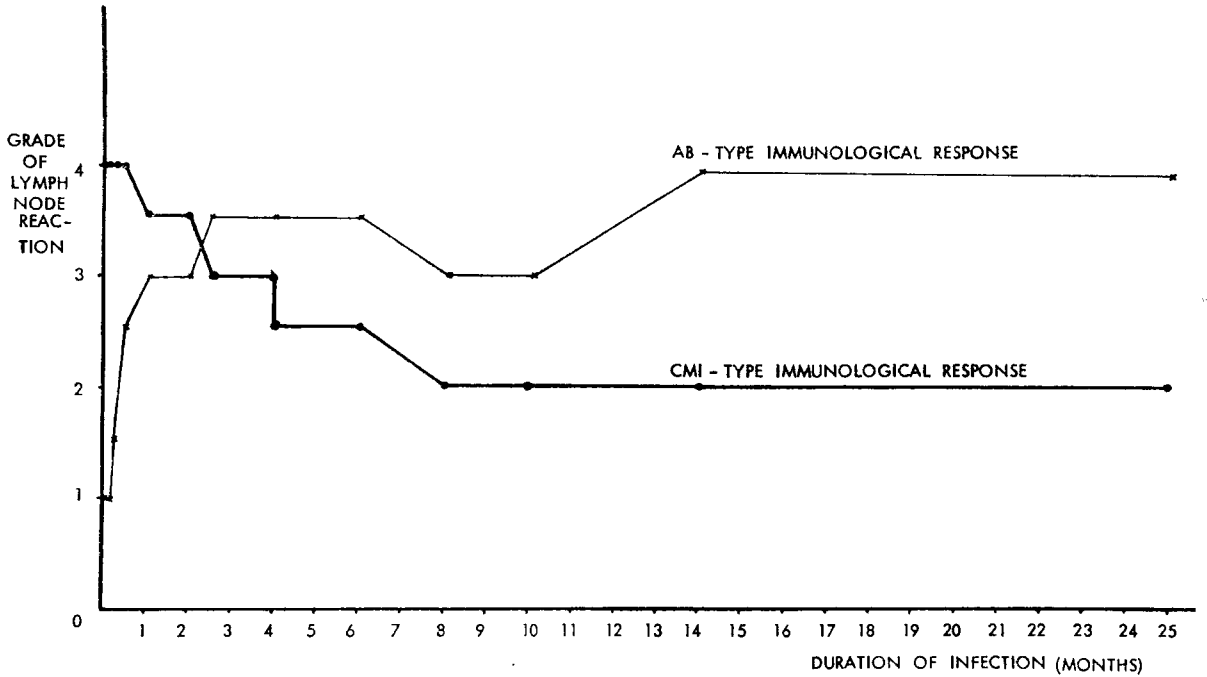


FIG. 8. Lymph node responses in cats infected with *B. pahangi* (Rogers *et al.*, 1975b).

gitis with infiltration of polymorphs and an accumulation of plasma cells, eosinophils and neutrophils around the infected vessels. Even after the worms had migrated into the lymphatics there was only a mild inflammatory response in the walls of the vessels and thickening of the valves near the worms. One month after infection there was a large increase in the diameter of the lymphatics with marked lymphangitis. The valves were thickened, extended and sometimes duplicated. The vessel walls were oedematous and there was an increasing acute inflammatory reaction with neutrophils, eosinophils and macrophages in the vessel wall and lumen and in the surrounding connective tissue. There was also an increase in adipose tissue beneath the muscle layers of the vessel wall. Fibrous tissue had developed in the outer two layers of the lymphatic wall.

From this time, lymphatic varicosity increased enormously with pockets of worms extending from the popliteal node to the tarsal joint. From approximately 8 weeks there was little change in the amount of lymphatic involved in the gross reaction. Histologically, the lymphangitis changed from an acute to a chronic reaction. Extra blood vessels developed around the lymphatics. Adipose tissue increased in the walls of the lymphatics.

Thrombolymphangitis was noted in older infections. The valves were thickened and their bases greatly expanded with plasma cells and polymorphs. Lymphoid follicles developed in the vessel wall or extended into the lumen, some with organised germinal centres which contained large pyroninophilic lymphoid cells. The lymphatics became partially occluded by fibrous and granulomatous lesions associated with the dead and disintegrating worms which were gradually replaced by organised fibrotic lesions (containing eosinophils and plasma cells), which in some cases appeared to block the vessel lumen completely. These fibrous lesions gradually resolved and cats which had been infected for 4 or 5 years often showed much less disorganisation of the lymphatics than did cats infected for a year or so.

Ah and Thompson (1973) studied the changes in lymphatics of jirds infected with *B. pahangi*. Most of their histopathological observations were made 3 months after infection when many of the changes described by Rogers and Denham (1974) would have occurred. Ah and Thompson (1973) stressed the changes associated with dead worms, which they found commonly in both lymphatics and nodes. The worms became encapsulated and invested with massive collagenous layers so that the lymphatic appeared blocked with fibrous reactions.

4. *Physiological reactions*

Rogers and Denham (1975) investigated the flow of material through normal and parasitised lymphatics. They injected colloidal radioactive gold ($^{198}Au$) subcutaneously into the feet of cats and plotted its rate of disappearance (Sage *et al.*, 1964). The gold disappeared equally well from the feet of limbs with *Brugia*-induced lymphatic damage and from the feet of normal limbs. However, one cat with an oedematous foot had a much slower movement of gold from this infected limb than from the foot of the normal contralateral limb. In cats, lymphoedema occurs only when they have become

microfilaria-negative (Rogers and Denham, 1974) and have developed an antibody which reacts with microfilarial antigen (Ponnudurai *et al.*, 1974). It is obvious that the cause of filarial oedema is complex, but it may be associated with the development of an immune response.

## VI. Immunology

Our understanding of the immune response to filarial infection lags far behind that of many other medically important parasites. Immunologists have been unable to study filarial infections because of the lack of suitable laboratory systems. To make any real progress in filarial immunology, systems using inbred rats and mice must be developed as a wealth of information has been gathered on the immune systems of these animals. Although a variety of filarial species infect rats in nature (Singh and Ho, 1973; Mullin and Balasingam, 1973), few of these have been established in inbred strains. It is a disadvantage that these rat parasites do not infect the lymphatics. However, both *Wuchereria* and *Brugia* have extralymphatic infective larval and microfilarial stages in their vertebrate life-cycles, and studies on these stages in other filarial systems may be relevant. Immune studies on infective larvae and microfilariae of any filarial species in an inbred system should be pursued. With this thought in mind, the following review on protective immunity will not be limited to *Brugia* spp., but will include encouraging results from other filarial and helminth systems.

### A. Innate immunity

The susceptibility of vertebrates to infection with *Brugia* varies between and within species (Sections II, IV). For example, studies in domestic cats have shown that Malayan cats have a higher rate of patency than do English cats. Unfortunately, the nature of this immunity in "resistant" individuals has not been analysed. Autopsies of these animals are rare and it is not known if resistance was directed against the developmental stages, adult worms or microfilariae. In many cases, viability controls are lacking and it is not known if the batches of third-stage larvae used were infective.

There are a few preliminary reports on the innate resistance of jirds to *Brugia*. Male jirds are more susceptible than females and the course of infection appears to be related to sexual hormones (Ash, 1973a; Section IVC). Wesley and Ash (Proceedings of Annual Meeting, American Society of Parasitology, 1972) showed that gonadectomy and injections of testosterone considerably altered the patency rates. The patency rates of cats do not vary according to sex (Denham, 1974a).

The existence of innate immunity to *Brugia* in man is not clear. In clinical–epidemiological surveys non-patent individuals are always found. However, the immune status of these people is not known because their parasitological histories are lacking. It is also not known how frequently they are bitten by infective mosquitoes or if their resistance is acquired or innate. In view of recent advances in our understanding of the genetic basis for susceptibility of verte-

brates to parasitic infection (Bradley, 1974; Wassom *et al.*, 1974; Allison, 1975; Wakelin, 1975), a careful analysis of resistance to filarial infection may be fruitful.

### B. ACQUIRED IMMUNITY

1. *Immunity stimulated by previous infection*

Denham *et al.* (1972b) repeatedly infected cats every 10 days by injecting 50 infective *B. pahangi* larvae into their left hind foot and found that some animals suddenly lost their circulating microfilariae. These studies have now been extended to include the stage-specific response of repeatedly infected cats (Denham *et al.*, unpublished). Cats that had received large numbers of repeated infections were divided into three groups according to their parasitologic status and challenged with infective larvae. The cats of Group A were challenged while they were still patent. Those of Group B were challenged soon after they became microfilaria-negative. Group C was challenged 6 to 9 months after they had become microfilaria-negative.

The cats which had received up to 50 repeat infections and still had circulating microfilariae (Group A) were susceptible to challenge. However, a few individuals of this group which had received more than 50 infections and had declining microfilaraemias were resistant to challenge but had live adult worms in their lymphatics. The immune status of these cats was probably transitional between Groups A and B.

The cats that were killed soon after becoming amicrofilaraemic (Group B) had live adult worms, from the repeat infections, in their lymphatics and microfilariae in histological preparations of their popliteal nodes. Although they had live adults in their lymphatics, they were resistant to challenge by infective larvae. While 50–60% of the challenge worms were recovered from the controls, a recovery of 1% was considered high in the experimental animals. The fate of the larvae inoculated 1 day before autopsy was very puzzling. Despite careful examination of lymphatic material no larvae were found. It was concluded that the larvae had been killed before reaching the lymphatics.

The cats which had been free of microfilariae for several months (Group C) were resistant to all stages of filarial worms. Only 1% of the challenge worms were recovered and living adults were not found in the lymphatics. Two of these cats were injected intravenously with microfilariae which were detectable in the peripheral circulation for 1 h. These cats suffered from an anaphylactic reaction. The control cats did not suffer from anaphylaxis and the microfilariae circulated for 3 weeks.

Resistance in the above animals was probably the result of acquired immunity and of pathologic changes in the lymphatics. Recoveries from the left hind limb, which was repeatedly infected, were usually lower than those of the other three limbs and the degree of resistance was often proportional to the degree of lymphatic damage. However, resistance was observed in "normal" limbs in some cats of Group A and all of those of Groups B and C, and it is likely that this form of resistance results from acquired immunity. The results of these experiments demonstrate that cats can develop an immune response

against all of the vertebrate stages of *B. pahangi* after continued antigenic stimulation over long periods.

Kowalski and Ash (1975) and Suswillo (unpublished) studied the resistance of jirds (*Meriones unguiculatus*) previously infected with *B. pahangi*. Kowalski and Ash (1975) inoculated jirds subcutaneously and produced a moderate degree of immunity. For example, jirds killed 130 days after the inoculation of 75 larvae gave a recovery of 16% whereas those given four infections of 75 larvae at weekly intervals gave a recovery of 9%. Another group given four infections of 20 larvae gave a recovery of 16% (cf. 15·5% of the $1 \times 75$ larvae group). Control jirds were not given 300 larvae in one inoculation so it is difficult to determine if resistance resulted from acquired immunity, pathologic changes or a crowding effect. Suswillo *et al.* (unpublished) inoculated jirds intraperitoneally with 50 larvae at weekly intervals for 5, 10 and 15 weeks. The percentage recovery of adult worms was the same for each group, suggesting that immunity did not develop after repeated infection. If the situation seen in cats is applicable to this model, it is possible that immunity might develop after further infection.

2. *Use of attenuated (irradiated) larvae*

Attempts to vaccinate with irradiated larvae have been successful in a variety of helminth systems and were highlighted by the production of three commercial vaccines: *Dictyocaulus viviparus* in calves (Jarrett *et al.*, 1959), *D. filaria* in sheep (Jovanovic *et al.*, 1965) and *Ancylostoma caninum* in dogs (Miller, 1971, 1974).

The principle underlying the use of irradiated infective larval vaccines is to stimulate immunity with the early stages of the parasite that are of low pathogenicity. Worms are most often attenuated by exposing them to ionising radiation. The effects of radiation on worms are dose-dependent and result in stunted growth and retarded development. Since development is retarded, the longevity of each larval stage is increased. If these stages secrete important protective antigens, irradiated larvae may stimulate a stronger immunity than normal larvae (Miller, 1966). Although early filarial larvae are the least pathogenic stages of *Brugia*, there is little evidence to suggest that they induce protective immunity. Studies on stage-specific immunity are urgently needed and the use of larvae attenuated with various doses of irradiation will be of great value. Wong *et al.* (1969) conducted the first filarial vaccination experiments using subperiodic *B. malayi* in *Macaca mulatta*. They varied both the number of larvae and the dose of X-irradiation. Two inoculations of 200 larvae attenuated with 20 krad stimulated protective immunity which lasted for at least 10–12 months. Their criteria for protective immunity were the appearance, level and duration of microfilaraemia. The vaccinated animals never became patent but had cryptic infections. Some monkeys were killed and examined for adult worms; one experimental monkey was negative and the other had a male and a gravid female while the controls had 4 and 11 adults of both sexes.

The induction of immunity against developing larvae and filarial adults by irradiated vaccines has been demonstrated with both *B. pahangi* and *Dirofilaria immitis* in dogs. Ah *et al.* (1974a) vaccinated dogs with 500 infective *B. pahangi*

larvae attenuated with a sterilising dose of 22·5 krad of gamma radiation. While all six of the vaccinated dogs became patent, four of them had suppressed microfilaraemias. The worm burden of the vaccinated dogs was reduced by 57% and two of the six dogs were not infected with adults. Even better results have been obtained with *D. imimtis* in dogs vaccinated with one or two injections of 100 larvae attenuated with 15 krad (Ah *et al.*, 1972). This dose did not sterilise all worms because some of the dogs in the vaccinated control group became patent. However, none of the five vaccinated dogs became patent on challenge and they had 82% fewer worms than the controls. Wong (1974) and Wong *et al.* (1974) have extended and confirmed these results.

Attempts to immunise cats against *B. malayi* have not been encouraging. Ramachandran (1970a) used a variety of vaccination schedules including the ones that protected monkeys and dogs (Wong *et al.*, 1969; Ah *et al.*, 1974a), but was unable to demonstrate protective immunity. The failure to induce protection in cats against *Brugia* may be related to the number of larvae and the irradiation dose used in the vaccinating inoculations. In our department, P. Oothuman irradiated infective stage *B. pahangi* with 25 krad and vaccinated with a total of 2100 larvae given on seven occasions. After challenge, the total worm burden of the five vaccinated cats was reduced by 60% compared with the controls.

The difficulties in inducing immunity in cats compared to dogs and monkeys illustrate that the immune response of each species is unique and that there are dangers in extrapolating results from one system to another. Until the data are collected, one can only hope that the human response to filarial vaccination mimics that of the monkey rather than the cat.

3. *Immunity stimulated by somatic antigens*

There have been many attempts to induce resistance to helminth infection by injecting homogenates or extracts of various worm stages but these attempts have usually failed (Thorson, 1970). Filarial nematodes are no exception to this trend (Feng, 1937; Krishnaswami and Pattanayak, 1959; Ramachandran, 1970a). Although protective antigens may be present in worm homogenates they may fail to induce resistance because of immune tolerance or antigenic competition.

Tolerance is the phenomenon in which animals produce an immune response to optimum doses of antigen, but fail to respond when exposed to too little or too much antigen (Roitt, 1974; Howard and Mitchison, 1975). Stimulation with the proper antigenic dose is so important that immunity has been described as an island in a sea of tolerance (N. A. Mitchison). Parasitologists have often injected large quantities of antigen using the "more the merrier" principle and it is quite possible that these large doses were tolerogenic. Recent observations in other systems indicate that low doses of worm homogenate can be immunogenic. Rothwell and Love (1974) induced protective immunity in guinea-pigs against *Trichostrongylus colubriformis* by injecting 100 $\mu$g and 1000 $\mu$g of worm homogenate protein. It would be worthwhile to include dose-response experiments in future attempts to vaccinate against filarial worms.

Antigenic competition may also play a part in the failure to induce immunity

with whole worm homogenates. Animals respond well to antigens when they are injected separately at different times, but respond poorly when they are mixed and injected together (Roitt, 1974). When protective antigens compete with other antigens in the homogenate for immune recognition, they elicit a weak response or no response at all. The development of dead helminth vaccines may be largely a problem of isolating protective antigens in pure form. For example, Wakelin and Selby (1973) found that antigens prepared from the anterior portion of *Trichuris muris* induced better immunity than antigens from whole worms or the posterior end. It is likely that the protective antigens present in stichocyte cells of the oesophagus cannot induce immunity in competition with determinants from the posterior portion of the worms. Similar studies on filarial nematodes may be fruitful.

Antibodies against the cuticle of third-stage and adult *Brugia* have been demonstrated by the fluorescent antibody technique (Ponnudurai *et al.*, 1974). However, there is no evidence that either immune sera or cells can kill nematodes through this cuticular "coat of armour" (Thorson, 1970; Ogilvie and Jones, 1973; Pappas and Read, 1975) and these anti-*Brugia* antibodies may not influence the infection.

Ponnudurai *et al.* (1974) also demonstrated antibodies against the sheaths and cuticle of microfilariae. Since the survival of microfilariae presumably depends on the transport of nutrients and excreta across the sheath and cuticle (Simpson and Laurence, 1972; Laurence and Simpson, 1974), these vital structures could well be susceptible to immune attack. It is noteworthy that microfilarial antibodies appear only after microfilariae disappear.

4. *Excretory/Secretory (ES) antigens*

Immunity against some nematodes has been stimulated by using material collected from worms kept *in vitro*. It is likely that this immunity is stimulated by ES antigens produced by metabolising worms. The effector arm of the immune response acts against these antigens to disrupt some vital physiologic function in the worm. Protective ES antigens are often related to the gut of nematodes and, in particular, to the oesophageal glands (Ogilvie and Jones, 1973).

There has been only one attempt to induce protection using ES products of filarial nematodes. Wong *et al.* (1969) incubated infective-stage *B. malayi* in physiologic saline at 37°C for 1 h. This "saline" failed to induce protective immunity in monkeys. Ah (pers. comm.) cultured infective *Brugia* larvae *in vitro* and found that they did not release ES products which could explain the failure of Wong *et al.* (1969). However, when Ah placed larvae in the peritoneal cavity of jirds for 1 day before culturing them *in vitro*, they grew from the third to the fourth stage and released ES products. This culture technique may be valuable in the collection of ES antigens in future immunologic studies.

5. *Evasion of the immune response*

Denham *et al.* (1972a) suggested that *B. pahangi* in the afferent lymphatics of the limbs occupy an immunologically deficient site as afferent lymph contains ittle antibody and few immunologically competent cells (Hall, 1967; Hall *et*

*al.*, 1969). However, the longevity of *Brugia* is not dependent on "privileged sites" because it survives quite well in the blood vessels and peritoneal cavity of the jird. In addition, other filarial nematodes infect a variety of tissues which cannot be considered "privileged sites".

Schistosomes evade the host's immune response by disguising their tegument with host antigens (Smithers *et al.*, 1969; McLaren *et al.*, 1975). McGreevy *et al.* (1975) attempted to show that *B. pahangi* protects itself in a similar fashion. Firstly, they attempted to demonstrate that infective third-stage larvae incorporated mosquito antigen on to their cuticles. Cats were immunised with thoracic muscle of *Aedes aegypti* and then challenged with third-stage larvae from this mosquito. There was no difference in the recovery of worms from the immunised and control cats although there was a delay in the migration of the worms back into the afferent lymphatics. The second approach was to immunise jirds against cat antigens and to transfer larval and adult worms and microfilariae from cats into these jirds. In each of these experiments there was no difference in the longevity or recovery of worms between anti-cat jirds and normal jirds. Attempts were also made to demonstrate mosquito or cat antigens on the surface of the worms by fluorescent antibody techniques. There was evidence for a small amount of *Ae. aegypti* on third-stage larvae but no mammalian antigen was found on the other stages in the life-cycle. It seems that *B. pahangi* does not evade the host immune response by disguising itself with host antigens.

6. *Effect of chemotherapy on resistance to infection*

In some helminth infections the host remains resistant to infection after removal of the parasites by anthelmintics (Campbell *et al.*, 1963; Campbell and Timinski, 1965; Denham, 1966, 1969). If this were true for human filarial infections there would be even more sense in using chemotherapy to control the infection. This point has not been properly investigated in areas where DEC has been used to control Brugian filariasis. Denham (unpublished) has been investigating this situation in cats infected with *B. pahangi*. Two cats have been treated with an anthelmintic and challenged six times and on each occasion a patent infection of the same level as the primary infection has resulted.

## VII. CHEMOTHERAPY

### A. THE CURRENT SITUATION

The only anthelmintic which has been used to treat *Brugia malayi* in man is DEC. Wilson (1950) hospitalised 25 symptomless, microfilaraemic patients and treated them with 2·0 mg DEC/kg body weight three times a day for 16 or 21 days. Treatment had a most dramatic effect and after the first day the mean microfilarial counts had fallen from 100/60 $mm^3$ to only 5/60 $mm^3$ (Fig. 9). Although Santiago-Stevenson *et al.* (1947) treated *Wuchereria bancrofti*-infected patients without serious side-effects, Wilson found very severe side-reactions in his patients after chemotherapy with DEC. Figure 10 shows the body temperature of two patients after treatment. Apart from pyrexia, most

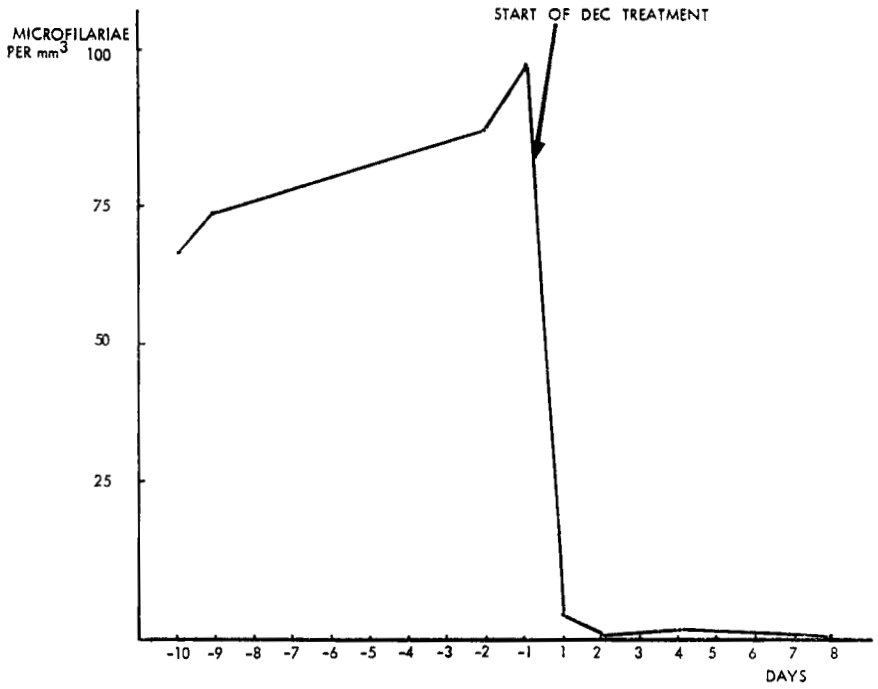


FIG. 9. The effect of diethylcarbamazine treatment on microfilarial levels in human patients with Brugian filariasis (Wilson, 1950).

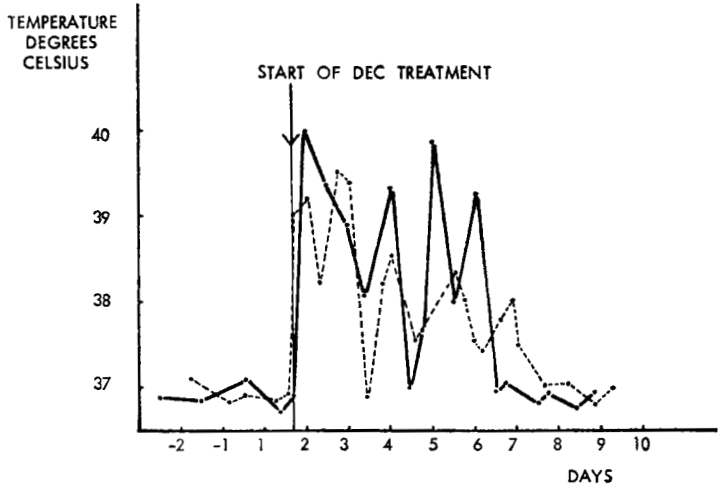


FIG. 10. The effect of diethylcarbamazine treatment on body temperature in patients with Brugian filariasis.

patients suffered vomiting, painful lymph nodes, painful joints in the lower limbs and headaches. The effects were reduced after a few days of treatment. Wilson examined several of these patients a year later and found that the majority were free of microfilariae. Most of those patients who had suffered from clinical filariasis before treatment reported a considerable improvement in their physical condition and no subsequent attacks of filarial fever. Turner (1959b) also hospitalised a group of patients and studied their reaction to treatment with DEC; he used 0·25–2 mg/kg once daily and found essentially the same side-effects as did Wilson (1950). Turner (1959b) also found that the side-effects of treatment only occurred at the beginning of treatment and that increasing the dose of drug during the later stages produced no adverse reactions The speed with which these side-reactions occur suggests that they are probably due to death of microfilariae rather than adult worms. In a patient with 10 mf/mm$^3$ and a blood volume of 4 litres, 40 million microfilariae may be destroyed in about 24 h and this massive release of antigen may account for some of the symptoms described by Wilson (1950). Further evidence supporting this comes from the lack of such responses in patients with clinical Brugian filariasis but without microfilariae in their blood.

### B. IS THERE A NEED FOR A NEW FILARICIDE?

DEC must be given as a long course of treatment and experience obtained in the South Pacific in attempts to eliminate *W. bancrofti* by mass chemotherapy indicates that large numbers of people fail to complete the course. The more complicated the treatment and the worse the adverse side-effects, the greater the drop-out rate. DEC produces much more severe side-effects in the treatment of *B. malayi* than it does in *W. bancrofti* infection (Wilson, 1950). DEC is far from ideal and a new drug is needed. If the drug which replaced DEC were only macrofilaricidal, most of the side-effects seen after treatment of Brugian filariasis with DEC would be avoided. There would also be no resurgence of microfilaraemia as happens after treatment with microfilaricidal drugs. It is probable that microfilariae of *Brugia* spp. live for only a few weeks and that destruction of the adult population will cause a slow decline in the microfilaraemia (Ponnudurai *et al.*, 1975). Although Ch'en (1964) produced evidence that DEC kills adult worms, this probably only happens after the use of very high doses of drug or prolonged therapy at the low levels normally used in treatment or control campaigns.

Suramin, which kills adult worms and microfilariae, is used in the treatment of onchocerciasis but it is generally considered to be too toxic for use in the treatment of Brugian filariasis. As will be seen in a later section, a number of other drugs kill adult *B. pahangi* in experimental systems but most of these are also too toxic to be used in human patients.

### C. THE COMMERCIAL INTEREST IN THE DEVELOPMENT OF FILARICIDES

The chances of a commercial company being able to make a profit from a new filaricide are not very great unless the compound has other more profitable

uses. The cost of testing a compound of known efficacy before it can be placed at the disposal of field workers for use against filarial worms is very high. The potential customers for filaricidal compounds come from the developing countries and are thus the people least able to afford to pay for the new compounds. One solution to this economic problem would be for an international agency to pay for the costs of developing new drugs since acceptable profits are unlikely even for a company which produces the "perfect filaricide".

Very few pharmaceutical companies are conducting research on the chemotherapy of filariasis, and obtaining compounds for evaluation in experimental filarial screens has been difficult. Most companies are wary of allowing outsiders access to unpatented drugs, and this problem can only be overcome by the development of trust between the pharmaceutical companies and the teams concerned with research into filaricidal drugs.

Experimental drugs are usually available in very small quantities while most tests for filaricidal activity are prodigal in their use of compound. This problem can be overcome by limiting filarial screens to compound of known nematodicidal activity and using screens which require minimal amounts of drug (Section VIID). Virtually every company which has an active screen for nematodicidal activity has detected a large number of compounds with activity against intestinal nematodes, but this activity is rarely good enough to warrant their development for commercial use. We believe that these drugs are the most likely candidates for filaricidal activity and they should be selected for screening. In addition to the development of new drugs, all anthelmintics that are already licensed for use in man should be evaluated for their effects upon filarial worms. Our survey of the published literature suggests that this is seldom done in experimental models, let alone in human patients.

## D. POSSIBLE SCREENS FOR FILARICIDAL ACTIVITY

In this section we discuss the methods we have developed to use *B. pahangi* in an economical way for filaricidal screening.

### 1. In vitro *screening*

Three stages of the life-cycle have been used for an *in vitro* screen.

(a) Adult male and female worms, dissected from the lymphatics of infected cats or recovered from the peritoneal cavity of jirds, are washed in Medium 199 containing antibiotics. For control cultures, worms are transferred individually to sterile, clear polythene tubes, containing 1 ml of the above medium plus 10% horse serum. For test purposes, worms are placed in various concentrations of the compound under investigation. The tubes are sealed, maintained at 37°C and observed at frequent intervals using an inverted microscope.

(b) Third-stage infective larvae obtained from *Aedes aegypti* are washed several times in Medium 199 plus antibiotics and placed in each well of a microtitre tray containing culture medium or various concentrations of test compound. The trays are covered to prevent evaporation and the cultures maintained and observed in the same way as those of adult worms.

(c) To obtain microfilariae, blood from a cat with a high microfilarial level is passed through a 5 $\mu$m mesh Nuclepore membrane filter held in a Swinnex filter-holder and the filter is washed with Medium 199. The filter is placed in a tube containing culture medium, which is agitated to dislodge microfilariae from the filter. Alternatively, microfilariae are washed from the peritoneal cavity of jirds inoculated by this route. A drop of the concentrated microfilarial suspension is placed in each well of a microtitre tray and culture medium or various concentrations of test compound added. The cultures are maintained and observed as above.

The worms survive remarkably well considering the simple media being used. Long-term observations on worms in test compound cultures are not necessary. Control cultures of adult and third-stage worms in polythene tubes are viable for at least a week, indicating that, from the physiological viewpoint, the system is satisfactory for the day or two needed to test the effect of the compounds.

Rogers and Denham (1976) used these techniques to study Levamisole. Adult worms in cultures with Levamisole concentrations below 0·015% coiled and moved actively during the 24 h of observation and survived after this. Concentrations of 0·5 and 1·0% inactivated all worms in 5 min and they did not regain motility when washed in fresh medium 18 h after exposure. Third-stage larvae were immobilised by higher concentrations. The speed with which they became immobilised was dose-dependent. Third-stage larvae were rapidly immobilised at 0·5% in 30 min whereas low concentrations took much longer (i.e. 0·05% took 18 h.). Below 0·04% there was no effect. Similar results were found using microfilariae.

We have also used these techniques to study DEC and Suramin. The results of these experiments are of special interest as they are the only two drugs used in man to treat filarial infection. Both were virtually inactive *in vitro* at physiological concentrations against all stages but very high concentrations killed the worms. Rogers and Denham (unpublished) tested a group of compounds of known filaricidal activity in animals or man and a group of nematodicidal compounds in the *in vitro* systems. Apart from DEC and Suramin, nearly all the filaricides were active *in vitro* as were about a third of the nematodicides.

2. *Screening in the insect vector*

Gerberg *et al.* (1966) and Gerberg (1971) studied the effect of various antimalarial drugs by feeding them to infected mosquitoes. Gerberg *et al.* (1972) adapted this technique to the study of filaricides and observed the effect of Levamisole on *Dirofilaria immitis* larvae in *Ae. aegypti*. Rogers *et al.* (unpublished) used this technique for screening for filaricidal activity against *B. pahangi*. Freshly infected, fully engorged female *Ae. aegypti* were transferred to small containers and provided with 10% sucrose solution in which was dissolved or suspended an appropriate amount of the drug. Ten days later, the mosquitoes were dissected and the filarial worms counted. Levamisole was the first compound to be tested in this screen (Rogers and Denham, 1976). It effectively reduced the number of infective larvae recovered by approximately 90% in mosquitoes exposed to 300 or more p.p.m. in a dose-dependent manner.

Rogers *et al.* (unpublished) also tested a group of 30 experimental nematodicides in this system. Fourteen of these compounds were 100% effective in preventing the development of infective larvae and a further five reduced the number of larvae recovered by half. Activity below this level was not considered significant. If this group of compounds is in any way representative of the numerous antinematode agents available, this screen will produce a surfeit of active compounds which will need further investigation.

3. *Screening in mammalian hosts*

Whatever *in vitro* or insect screens are used, active compounds must be tested in animal models.

(a) *Jirds.* Since the reports of Ash and Riley (1970a,b) that the jird was a good host for *Brugia* spp., many people have been using this system. Several reports have been made in abstract concerning the activity of DEC. In most cases the drug had microfilaricidal activity, but little or no activity against adult worms even though very large doses of drug were used. Denham *et al.* (unpublished) tried to demonstrate activity of DEC, using *B. pahangi* infections in jirds. They initially used the drug at 25 or 300 mg/kg on five consecutive days but could not detect significant activity.

Like other workers (Section IVc), we have found that subcutaneous infections give very variable infection rates and we prefer to use intraperitoneal infections for chemotherapeutic studies. Even with these, it was difficult to determine if DEC had any activity against adult *B. pahangi*. A new system for evaluating potential filaricides in jirds was, therefore, developed. In this new test, a few jirds are infected intraperitoneally with large numbers of *B. pahangi* larvae and left for 75 days when adult worms are recovered from their peritoneal cavities (McCall *et al.*, 1973). The adult worms are immediately anaesthetised in 1% Methyridine (ICI Pharmaceuticals Ltd), which prevents them tying themselves in an inextricable ball. Known numbers of male and female worms are transplanted into the peritoneal cavities of a large number of recipient jirds (Butts and Rabalais, 1974). This technique produces highly reproducible infections. Compounds can be tested a few days after the recipient jirds have been infected. Under these circumstances, DEC at 300 mg/kg for 5 days had no effect upon the adult worms.

One of the major problems in using an animal model for testing compounds is the cost of animal care during prepatent periods. Drugs cannot be screened against *Brugia* for at least 75 days after infection, and during this period valuable animal accommodation is being occupied while money is expended on food and maintenance. In the new test, very few animals have to be retained for long periods. Once the worms are mature in the donor jirds, large numbers of jirds can be used as recipients, treated 2–3 days later and killed in 10–14 days. Thus, with limited animal accommodation, at least five times more tests can be conducted than by conventional means. The reproducibility of the test is such that only two animals need be used for each test compound.

(b) *Anthelmintics in cats with* Brugia *infection.* One of the long-term experiments being conducted in this department has been the testing of a series of anthel-

mintic compounds against *B. pahangi* infection in cats. Edeson and Laing (1959) and Denham *et al.* (unpublished) found that DEC killed adult worms but not microfilariae of *B. pahangi* and *B. malayi* in cats. Once again we see that DEC has an effect which would not have been predicted.

Denham *et al.* (unpublished) tested the effect of Suramin on *B. pahangi* in cats and found that the adult worms died some 5 weeks after treatment. Hawking (1955) originally stated that Suramin had no effect upon *Litomosoides carinii* infections but it has since been shown (Lammler *et al.*, 1971; Stohler, unpublished) that adult worms die about 2 months after treatment.

This finding has serious implications for the design of a screen for filaricidal activity, as one would want to kill and examine animals as soon as practical after treatment and keeping animals for an extra 2 months would be extremely expensive. If animals were killed a month after treatment, Suramin would be judged inactive. One must therefore make a decision on the duration of the screen being used. Does one accept that the screen will not detect the filaricidal activity of Suramin or does one leave the test animals in the laboratory for longer periods than seem necessary for other drugs to kill filariae? To our knowledge, Suramin is the only filaricidal compound which fails to kill worms by 1 month and we now terminate our screens at this time.

Levamisole is a very good microfilaricide against *Brugia* in cats (Mak *et al.*, 1974; Rogers and Denham, 1976) and this seems to agree with the limited knowledge of its action on *B. malayi* in man (O'Holohan and Zaman, 1974). There may be a difference in the response of *B. malayi* and *B. pahangi* in cats. Mak *et al.* (1974) found that Levamisole killed adult *B. malayi* in cats, whereas Rogers and Denham (1976) found that it was less effective against adult *B. pahangi*.

Drug trials against *Brugia* spp. in cats are expensive. We have been using this system for several years and have adopted the following scheme. Both hind legs of cats are infected with *B. pahangi* larvae. When they have been patent for about 2 months they are treated with the test compound for 5 consecutive days. Microfilarial counts are made throughout the course of treatment and weekly thereafter. A biopsy of the popliteal afferent lymphatic of one leg is taken 7 days after treatment and the cat killed 21 days later. Usually two cats are treated with each drug. Control cats are not killed. This cuts the cost of the test by 50%. Experience has shown that in an occasional cat adult worms die spontaneously but this is so rare that control animals would be of little value. As dead worms can be seen in the lymphatics, it is possible to make a reasonably accurate estimate of the total worm burden before and after treatment. Most drugs tested in this system are either completely inactive or completely active so that one either finds large numbers of living worms or only dead and decaying worms.

### E. ACTIVITY OF DEC IN SCREENS

When developing new screens for anthelmintic activity it is important to use a series of reference compounds of known activity to compare the efficiency of the screens. Unfortunately, very few compounds are active against human filariae. The most obvious reference compound to evaluate screens for filaricidal activity is DEC.

When the activity of DEC was first discovered, using *L. carinii* in the cotton rat, it was reported that the drug was macrofilaricidal (Hewitt *et al.*, 1947) but this initial observation has not been confirmed (Hawking, 1955; Stohler, pers. comm.). DEC is a potent microfilaricide in man but there is still controversy about its effect on adult worms. After high doses or long-term treatment, the drug kills adult *W. bancrofti* or *B. malayi* (Ch'en, 1964). It is most unlikely that DEC would have been developed if it had been known that it was only microfilaricidal in *L. carinii* infections; especially as DEC was only one of a group of compounds reported to have macrofilaricidal activity.

In *in vitro* tests and in the insect test DEC has little, or no, activity. In the jird it may have microfilaricidal activity but it is extremely doubtful if it kills adult worms. In the cat it has little effect on microfilariae but has good macrofilaricidal activity. If one accepts that primary screening for new compounds will not be carried out in the cat, it is doubtful if any test currently being used for primary screening would detect DEC; this includes *L. carinii*, as one is, presumably, searching for a macrofilaricide.

If one accepts that there is no satisfactory reference compound, one should use the cheapest possible test to select compounds for further evaluation. It is recognised that by undertaking this arbitrary elimination process one will be rejecting some potentially useful compounds, but if the sample of 30 compounds used by Rogers and Denham (unpublished) *in vitro* and if the insect test is any guide, many active compounds will be found. The question is what to do after an *in vitro* test.

The new test in jirds which was described above has potential as a secondary screen owing to its relatively low cost and reproducibility. After this a tertiary screen could be performed in cats and any satisfactory drugs tested in man after the necessary toxicity tests.

## VIII. Control of Brugian Filariasis

For the sake of brevity we will consider only the rationale behind control. We are aware that the application of any technique is complicated and involves social, logistical and managerial considerations. These are beyond the scope of this review and the reader should consult the Third Report of the Expert Committee on Filariasis (WHO, 1974), Hawking (1974) and Ramachandran (1970b,c).

There are two general approaches to the control of vector-borne diseases. In the chemotherapeutic approach, transmission is broken by eliminating man's infectivity to the vector, while in the entomological approach transmission is stopped by removing the vector's infectivity to man. Both approaches have been used to control Brugian filariasis. Mass chemotherapy with DEC has been used to reduce man's infectivity to mosquitoes and the application of insecticides and larval control have been used to reduce mosquitoes' infectivity to man.

### A. Control of periodic *Brugia malayi*

The control of periodic *Brugia malayi* is straightforward because it is

almost host-specific to man in nature. This parasite is nocturnally periodic and is transmitted by night-biting mosquitoes. Since people sleep at night, most transmission occurs in the bedrooms. To deny mosquitoes access to sleeping humans is one obvious way to control filariasis and this can easily be accomplished with mosquito nets. To our surprise, we are unable to locate any information on the impact of mosquito nets on the transmission of any vector-borne disease. Nor can we find any literature evaluating the effects of "mosquito proofing" the sleeping rooms. However, there is a current investigation in Tanzania to find the cheapest way to modify the bedrooms of mud huts to block the entry of mosquitoes (Kolstrup, pers. comm.). Preliminary results are very encouraging and show that the biting intensity of *Culex p. fatigans* can be reduced from 200 to five per person per night simply by installing a door, a ceiling and window-screens. It is most significant that these modifications can be made by unskilled labour using local materials at a *per capita* cost that is competitive with more conventional techniques to control mosquitoes. It is not known if such modifications will stop filarial transmission, but it is known that Bancroftian filariasis is more prevalent in "bad" huts than in "good" huts (Wijers and Kolstrup, pers. comm.).

Since transmission occurs in bedrooms, the vectors can be attacked with residual insecticides. The effect of residual sprays on a mosquito population is directly related to the behaviour of the target species. Many *Anopheles* spp. prefer to feed on sleeping humans and rest in bedrooms where the application of residual insecticides may reduce or eliminate the entire mosquito population and break filarial transmission. Not all vectors are as dependent on human blood for their survival and feed indoors and outdoors on a variety of animals. The application of residual insecticides to bedroom walls has little impact on the total population size of these vectors. Wharton and Santa Maria (1958) have shown that the vast majority of the *Mansonia* vectors which enter houses to feed are killed by the insecticide, but that the elimination of this small segment of the total mosquito population had little effect on the human biting index. However, transmission of nocturnally periodic *B. malayi* may still be broken because this parasite has no functional animal reservoir and all mosquitoes that become infected from man succumb to the lethal effects of the insecticide.

In many endemic areas, larval control may be the method of choice against *Mansonia* if breeding is limited to man-made ponds and tanks. Optimal control may be obtained by using herbicides to eliminate the water plants which provide air for the mosquito larvae (Chow, 1953; Chow *et al.*, 1955). The anopheline and aedine vectors usually breed throughout vast areas which makes the control of their larvae a most difficult job.

Theoretically, it is possible to break the transmission of periodic *B. malayi* with vector control, but there is little data to evaluate the efficacy of this technique in the field. The reason for this deficiency is the general acceptance that *Brugia* infections are long-lived and that it would be biologically impossible and both economically and ecologically prohibitive to suppress vector populations long enough for all patent carriers to turn negative. Although this hypothesis may be true, it is not supported with facts as absolutely nothing is

known about the mean longevity of the patent period of *B. malayi* in humans in the absence of continual transmission.

Examinations of people at various intervals after leaving areas endemic for Bancroftian filariasis indicates that the maximum patent period for this parasite may be around 8 years (Jackowski *et al.*, 1951; Leeuwin, 1962; Mahoney and Aiu, 1970) while the mean longevity of adult worms is estimated to be 2–4 years (Hairston and De Meillon, 1968). Similar information on *B. malayi* is needed to evaluate the potential of vector control. The impact of vector control on periodic *B. malayi* should be carefully evaluated in the field.

In the past 25 years there have been many attempts to control the vectors of malaria and filariasis throughout the East, but precise information on the methodology and results is piecemeal. Perhaps the most notable of control programmes was conducted in Sri Lanka from 1947–57. Surveys in 1964/65 showed that *B. malayi* was nearly eradicated (Abdulcader, 1967; Dissanaike, 1969; Hawking, 1973). The major antifilarial control measure was the treatment of ponds with herbicides to kill *Mansonia* larvae. Unfortunately, the actual effects of larval control are clouded by the simultaneous application of residual insecticides for *Anopheles*/malaria control and the use of DEC.

The chemotherapeutic approach to the control of Brugian filariasis has received considerable attention. There are two main approaches: the "individual treatment" of positive cases and "mass treatment" of entire populations. Since the methods of detecting microfilariae used in most areas are inefficient, a sizeable proportion of the population with low densities of microfilariae which are still infective to mosquitoes will be missed and not treated if individual treatment is undertaken. Individual treatment also promotes a sense of embarrassment in the people involved, resulting in reluctance to accept treatment (Ramachandran and Dondero, 1973). On the other hand, mass chemotherapy encourages a sense of community action among the population which helps to reduce the proportion of individuals who refuse therapy or fail to complete a full course of treatment. The initial thrust in a DEC control programme should be made with mass chemotherapy. Individual therapy should be restricted to carriers detected in surveillance operations.

The initial field trials to control Brugian filariasis by mass chemotherapy were conducted in West Malaysia by Edeson and Wharton (1958a), Wharton *et al.* (1958) and Turner and Sodhy (1959). Considering the side-effects of treatment (Section VIIA), the authors of these reports are to be congratulated on the number of patients completing their course of treatment. Since these heroic pioneer studies, there have been a number of large-scale pilot experiments to study the use of DEC for the control of Brugian filariasis. Kim *et al.* (1973b) and Seo and Whang (1974) in South Korea, Harinasuta *et al.* (1970) in Thailand, Ramachandran (1970b) in West Malaysia, Sajidiman *et al.* (1975) in South Borneo and Putrali *et al.* (1975) in Sulawesi have all shown the great potential of this form of control. Programmes are also in progress in India and China (Hawking, 1973, 1974).

It is now established that a proportion of the population infected with either *B. malayi* or *Wuchereria bancrofti* continue to circulate microfilariae at den-

sities of 1–60 mf/ml of blood after taking a full course of treatment (Edeson and Wharton, 1958a; Turner and Sodhy, 1959; Southgate, 1974; Desowitz and Southgate, 1973; Sajidiman *et al.*, 1975). Therefore, control of transmission with DEC largely depends on the frequency that residual microfilariae are ingested by mosquitoes and develop to the infective stage. Since the amount of blood ingested by different mosquito species varies as does their susceptibility to infection, the threshold density of microfilariae below which transmission will cease varies from one endemic focus to the next. For example, *Aedes polynesiensis* and *C. p. fatigans* ingest microfilariae of *W. bancrofti* when fed on carriers with densities of 60 mf/ml but the smaller *Anopheles gambiae* not only ingest fewer microfilariae, but kill 50% of them with their pharyngeal and cibarial armatures (Section IIIB; Bryan and Southgate, 1976; McGreevy *et al.*, unpublished). *W. bancrofti* transmission continues in *Ae. polynesiensis* and *C. fatigans* areas after DEC mass chemotherapy and that careful surveillance and further treatment may be necessary for local eradication. Transmission is unlikely to continue in *An. gambiae* areas and local eradication may be achieved by chemotherapy alone. Little is known of the ability of *Mansonia*, *Anopheles* and *Aedes* to transmit the residual microfilariae of *B. malayi* (Sajidiman *et al.*, 1975). An analysis of these factors will provide valuable insight into the impact of DEC on transmission and could influence the strategy of future control programmes. It is conceivable that some vectors of *Brugia* fail to transmit residual microfilariae, making expenditures of funds and manpower on vector control unnecessary. Whether or not mass chemotherapy with DEC breaks transmission completely, it eliminates morbidity caused by Brugian and Bancroftian filariasis and its continued use is to be encouraged (Wilson, 1950; Bryan and Southgate, 1976).

### B. CONTROL OF SUBPERIODIC *BRUGIA MALAYI*

The control of subperiodic *B. malayi* with its strong domestic and feral zoonotic potential presents much more of a problem than does that of periodic *B. malayi*. In zoonotic situations, control by elimination of the vector is theoretically correct but the behaviour of *Mansonia* in the swamp forest makes this task impossible. These mosquitoes breed in enormous numbers over vast areas, and to destroy their host plants with herbicides or the larvae themselves with insecticides would be ecologically and economically prohibitive. The control of adult mosquitoes by using residual insecticides in houses would also be futile because they feed indoors and outdoors on animals as well as man.

The control of subperiodic *B. malayi* with DEC has not been successful because of the animal reservoir which serves as a source of infection for humans (Wharton *et al.*, 1958). However, chemotherapy has been limited to the human population and no attempt has been made to treat the domestic animals. Drugs which are more efficient than DEC are now available for use in cats and dogs and these should be used to eliminate the domestic animal reservoir. Not only would this eliminate the domestic source of infection but it would allow the role of the animal reservoir to be properly assessed.

### C. SURVEILLANCE

The success of a filarial control programme based on the interruption of transmission cannot be known for at least 10 years after the attack phase and surveillance operations should continue for this period of time. The length of this period is dependent on the longevity of the adult filariids and the extent to which transmission persists.

The status of transmission can be monitored by comparing the incidences of infection in both man and mosquitoes at various intervals. Although it would be most valuable to monitor the entire population, this is often impractical and necessitates the selection of an indicator group. Particular attention should be given to young people because the presence of carriers in this group after treatment is strong evidence for continued transmission. This assumption is less firm in older age groups because they harbour residual microfilariae from old infections in addition to microfilariae from new infections.

With full recognition of the problem of analysing incidence data from adults, it is still suggested that a sample of older men be included in surveillance after chemotherapy in regions with zoonotic Brugian filariasis. In the swamp forest, the younger people tend to stay within the kampong while men venture into the forest. Therefore, the incidence of infection in the young group will indicate the degree of transmission within the kampong while the incidence in adult males reflects transmission from the wild animal reservoir in addition to that of the kampong. Further analysis of kampong transmission can be gained from the incidence of infection in the domestic animal reservoir.

The prepatent period of *Brugia* and probably *Wuchereria* is around $2\frac{1}{2}$ months and fluctuations in the incidence of infection can only be detected over long periods of time. More immediate information on the success of a control programme can only be obtained from changes in the incidence of infection in mosquitoes.

Epidemiological studies of filarial worms in mosquitoes are limited to infection with microfilariae and infective larvae because these helminth stages can usually be identified as either human or animal parasites. Data relating to the developmental stages in the thorax are only valuable in areas where the vectors feed largely on man or where the enzootic filariae develop in mosquito organs other than the thorax.

The value of data on the prevalence of microfilariae or third-stage larvae in the vectors depends largely on the methods of mosquito collection and dissection. As an epidemiologist is only interested in those mosquitoes delivering infective larvae to man, the collection techniques should be limited to those using human bait. The entomological literature contains a wealth of information on a variety of trapping techniques which often capture large numbers of mosquitoes. In most cases it is not known if the numbers and species diversity of the trapped insects is related to those which bite man. Medical entomologists also question the validity of human bait catches, but there can be no doubt that they relate to natural biting behaviour better than result obtained with traps.

If the captured mosquitoes are to be dissected for infective larvae, it is

crucial to collect individuals which have not probed or fed on the human bait. Infective larvae commence their emergence from mosquitoes shortly after probing begins (McGreevy *et al.*, 1974b) and the intensity of infection determined from mosquitoes caught after this time would be misleadingly low. It is also likely that the infectivity rates would also be low because naturally infective mosquitoes most frequently harbour only one or two larvae. These mosquitoes would be scored as negative if the worms escaped before capture.

From an operational point of view, the determination of infectivity rates in a mosquito population is very time-consuming as it involves all-night bait catches. In general, infectivity rates are very low and frequently 1000 mosquitoes must be dissected before an infective individual is found. After DEC treatment it may be necessary to examine 10–20 000 mosquitoes before finding a positive. To dissect such large numbers is clearly beyond the capabilities of the control entomologist and other techniques to assess the impact of DEC on transmission are needed.

The objective of a DEC campaign is to reduce the infectivity of man to mosquitoes and it seems that the most sensitive entomological indicator of this phenomenon might be the incidence of microfilarial infectioni n recently fed mosquitoes. These mosquitoes can be collected easily from human habitations by a variety of simple techniques such as the hand catch, spray catch or window-trap methods. The bloodmeal and thoraces of the mosquitoes are macerated in separate pools of water and examined immediately for microfilariae under the dissecting microscope or stained and examined later. The incidence of microfilarial infection in mosquitoes collected from houses containing infected individuals may range from 10–20% in pretreatment surveys and should approach and remain at zero if control were successful.

### D. CONCLUSIONS

DEC is a difficult drug to administer and in mass chemotherapy programmes the proportion of the population who default on their dosage can be high as a result of side-reactions and the prolonged course of treatment. Even with minimal default rates, transmission may continue from carriers harbouring ultralow densities of persistent microfilariae. Until transmission ceases, control will rely on continual surveillance and individual treatment. There can be no question that a more practical drug is needed. Recent research indicates that there are a number of compounds with potential filaricidal activity, but research on their efficacy in man is minimal.

Just as useful as a new drug for treatment, would be a vaccine for prevention of infection. In spite of this need, there has been little research on filarial immunology and, consequently, there have been few developments. Although preliminary attempts to vaccinate with irradiated larvae have yielded promising results, the development of any vaccine is dependent on a major breakthrough in the mass culture of infective larvae from microfilariae *in vitro*. Based on estimates for the development of an irradiated dog heartworm (*Dirofilaria immitis*) vaccine in the United States, it will cost five million dollars to develop a *Brugia* vaccine and take at least 5 years (T. M. Miller, pers. comm.), a

cost which is similar to that required to develop a completely new anthelmintic. This expenditure can only be justified if the *Brugia* vaccine elicits heterologous protection against *W. bancrofti* and, most important, *Onchocerca volvulus* because these worms do not have animal hosts to obtain the necessary quantities of worm antigen to vaccinate the millions of people at risk.

Attempts to vaccinate against filarial nematodes and other helminths indicate that the advantages or immunisation over mass chemotherapy may be minimal. Just as most anthelmintics must be given on more than one occasion, so booster inoculations are required for optimal protection against helminths and other organisms. Drugs often cause side-reactions, but similar reactions may also result from the vaccination of hypersensitive individuals in a community. These reactions could restrict vaccination to the young and leave the old as reservoirs of infection. A major disadvantage of DEC is its failure to kill all microfilariae, but likewise, vaccination against helminths is often incomplete and a few worms often "slip by" to produce patent infections. Vaccines have the advantage of protecting over long periods, but relative to chemotherapy this advantage is of marginal importance if the parasite is eliminated from a community by breaking transmission with a proper mass chemotherapy programme. In endemic areas a high proportion of the public is already infected and it is this part of the community which demands treatment. The true value of a vaccine will be to prevent the reintroduction of filariasis into these areas after mass chemotherapy.

In addition to chemotherapy and vaccination, filariasis can be controlled by controlling its vectors. There are a battery of methods available and they generally involve the use of chemical or biological agents, genetic manipulation and environmental alterations. It was not within our scope to review the technological problems of applying the different methods of mosquito control nor their biological repercussions, and the interested reader should consult other reviews (Springett, 1975). However, we would like to stress two features which are typical of most methods of insect control. Their application is usually complex and requires a high degree of technical expertise and/or it is expensive. This expertise and money are often not available in countries with filariasis.

There is no question that in the long term mosquito control is superior to both chemotherapy and vaccination because it eliminates their pestiferous bites and all the diseases they carry. There is an urgent need to develop simple control methods which utilise local resources and local labour-techniques which can be used by ordinary people as part of their development programme based on self-reliance. Experimental data comparing the cost and impact of "mosquito proofing", "bed nets" and similar innovations with the classical types of control for filariasis and other tropical diseases are needed. Such methods will not eliminate mosquitoes but they will reduce their biting rates to tolerable levels—levels which may break transmission of inefficient parasites such as *B. malayi* or *W. bancrofti.*

Any effort to control mosquitoes by "self-reliance" must effect change in human behaviour through some motivational and/or educational programmes (Dunn, 1974). Vast amounts of information have been accumulated on the behaviour of mosquitoes and filarial worms, but the third component of the

life-cycle, man himself, has been neglected. To change man's behaviour is no easy task, but we must try. Studies on the social anthropology of target populations and the development of effective techniques in public health education and communication will be fruitful. The relationship between "self-reliance" and the control of vector-borne diseases has been developed in a most interesting article by Gillett (1975).

Unfortunately, this review does not bring good news to public health personnel in countries with endemic filariasis. New drugs, a vaccine, new techniques to control vectors and effective methods to change human behaviour will not be available for many years and it would be senseless to delay filarial control and hope for the development of a miracle cure. Therefore, the expansion of current programmes using DEC and vector control should be encouraged. Experience throughout the East and notably in Sri Lanka indicates that filarial disease and possibly the parasite itself can be eliminated by the proper application of current techniques. These isolated success stories lead us to believe that the major reason for our failure to control filariasis stems from the lack of true dedication. For example, Hawking (1973) reported that more than 100 prevalence surveys have been conducted in 21 major geographical areas endemic for Brugian filariasis, but that control procedures have been initiated in only ten of these areas. It is relatively easy to examine people for filariasis but it is hard work to control it. The control of filariasis in the future will depend on the number of public health personnel who are willing to accept the challenge and extend their work on prevalence and distribution to the realm of control.

## Acknowledgements

We are grateful for a grant from Hoffman–La Roche, without which this review could not have been prepared.

We have been supported by a project grant from the British Overseas Development Authority through the Tropical Medicine Research Board (D. A. Denham) and by the Wellcome Trust (P. B. McGreevy), which we gratefully acknowledge.

We thank Professor G. S. Nelson for his comments on the manuscript. We are especially grateful to Dr Rosemary Rogers for her help. Mrs Elizabeth Manson provided the careful typing which simplified our task. Miss Susan Vicary and Mr Pakeer Oothuman drew the figures.

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# Experimental Epidemiology of Hydatidosis and Cysticercosis

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## I. Introduction

The Taeniidae contain species that are responsible for serious health and economic problems. These problems have been investigated extensively in recent years.

Among the more important Taeniidae are *Echinococcus granulosus* and *E. multilocularis*. The biology of these species has been reviewed by Smyth (1964, 1969). A number of reviews record prevalence rates and geographical distribution in the various animal populations (Gemmell, 1960, 1961a; Simitch, 1961, 1962, 1964; Rausch, 1967; Schantz and Schwabe, 1969; Williams *et al.*, 1971). Although there is at least one ethological stituation where man remains an alternative intermediate host (Nelson and Rausch, 1963; Nelson *et al.*, 1965; Mann, 1974), he is normally regarded as an accidental host. Nevertheless, it is the hydatid organisms with their ubiquitous distribution that give rise to serious health consequences in almost all regions where suitable definitive and intermediate hosts co-exist.

Important cestodes responsible for cysticercosis in food animals include *Taenia saginata*, *T. solium*, *T. hydatigena* and *T. ovis*. The geographical distribution and medical aspects of *T. saginata* have been comprehensively reviewed by Pawlowski and Schultz (1972). Man is an obligatory definitive host for *T. saginata* and *T. solium* as well as an important anomalous intermediate host for *T. solium*. While usually the strobilate phase does not gravely affect human health, tapeworm infections are aesthetically unacceptable. The larval phase, however, of *T. solium* may affect health, and neurocysticercosis may be fatal. Limited surveys covering *T. saginata* and *T. solium* are recorded in the World Health Statistics Report (Anon., 1969). Man has been recorded as an intermediate host of *T. hydatigena* by Slais (1965) and of *T. ovis* by Abuladze and Sadykov (1969) and Sadykov (1971), but these are essentially parasites of dogs and sheep.

As meat hygiene standards improve, the hydatidosis/cysticercosis complex may provide formidable barriers to the expansion and to the development of trade. Legislation exists in most countries to control cestodes of public-health importance. In a few countries, control programmes against *E. granulosus* and *T. solium* have been effective. It is only recently that consideration has been given to controlling those species that contribute more to the loss of edible protein than to human health.

The premise can be constructed that advances in drug therapy, serodiagnosis and immunization should provide a wide choice of methods for the control of the hydatidosis/cysticercosis complex in a protein-hungry world. The most suitable control measures will depend upon the stability of the parasite population in an endemic zone. Effective control measures require a thorough under-

standing of epidemiology. Epidemiology is concerned with diseases in populations and embraces both the descriptive and experimental approach. The first is concerned with observations in the field and takes account of social factors. The second, and the subject of this review, is concerned with testing the various hypotheses that arise as a result of the observations.

There are problems in undertaking epidemiological research. This is because there are, as yet, no known small animal hosts suitable for experimental studies with larval *T. saginata* and *T. solium*. In addition, costly facilities may be required to work effectively with the normal hosts, especially in endemic regions. The sheep metacestodes *T. hydatigena*, *T. ovis* and, to a lesser extent, *E. granulosus* provide useful models for quantitative studies. This review describes the epidemiology of *T. saginata* and *T. solium*, but uses the sheep metacestodes as principal models in a systematic approach to an understanding of the factors that regulate larval tapeworm populations in the various ecological situations expected from a global distribution.

## II. Evolution of Regulation of Cestode Populations

Sprent (1962) developed the hypothesis that both parasites and hosts have undergone a series of reciprocal adaptations during evolution. As the specificity of the immune response evolved, the successful parasite adapted by changing its antigens to resemble more closely those of the host. The ultimate form of masking or mimicry should result in the evolution of identical antigens by both host and parasite. Natural selection for the parasite, however, should lead to the convergence only of those antigens concerned with stimulating the mechanisms of resistance in the host (Dineen, 1963a,b). Antigen-sharing between host and parasite may constitute convergent evolution (Damian, 1964). Antigen-sharing between parasites may have evolved to limit the abundance of competitive species in the normal host (Schad, 1966). With tapeworms, antigen-sharing may also have evolved so that larvae occupying different intermediate hosts can effectively occupy a common definitive host in the strobilate phase (Gemmell, 1969a). In the case of *Echinococcus*, Smyth and Smyth (1964) have pointed out that hermaphroditism of the adult and polyembryony of the hydatid organisms are conditions that favour the expression of mutants and their selection and establishment in different hosts.

Adaptations for mutual survival of parasite and host populations is an important feature in epidemiology. It has been postulated that when the parasite's biotic potential is well above the minimum required for transmission, regulation may be determined by the individual host (Bradley, 1972; Gemmell, 1975). Very wide changes in the parasite population can occur without prejudicing the survival of the host population. Animals are for various reasons not uniformly parasitized. Both intrinsic and extrinsic factors contribute to the infective pattern.

While evidence is presented in this review that strong density-dependent mechanisms have evolved to limit larval cestodes, little is known of the importance of these mechanisms in regulating adult taeniid tapeworm populations.

Certain analogies might be found among Hymenolepididae. In this family it seems that in order to avoid overloading the arthropod intermediate host, the principal regulatory mechanisms have evolved in the mammalian definitive host. For example, only a very small proportion of white-footed deer mice (*Peromyscus maniculatus*) failed to manifest an immune response to *Hymenolepis citelli*. Only these are responsible for disseminating eggs to the camel cricket (Wassom *et al.*, 1973, 1974). Further analogies between Hymenolepididae and Taeniidae may be found particularly in the relationships between the strobilate and larval phase of *H. nana* in rodents (Weinmann, 1966, 1970; Heyneman, 1963) and *T. solium* in man.

## III. Pathogenesis and the Infective Pattern of Larval Cestodes

In reviews on immunity to cestodes (Gemmell and Macnamara, 1972; Gemmell, 1975) and on their pathogenesis (Smyth and Heath, 1970), a number of problems concerned with interpretation of data have been discussed. Smyth and Heath (1970) point out that statements concerning pathology may only be applied to a particular "strain" of parasites in a particular "strain" of host. Studies on the growth and pathogenicity of *T. saginata* and *T. solium* (Viljoen, 1937; Slais, 1970), *T. saginata* (Penfold, 1937; McIntosh and Miller, 1960; Silverman and Hulland, 1961), *T. hydatigena* (Pullin, 1955; Sweatman and Plummer, 1957; Shepelev, 1961b), *T. ovis* (McCleery and Wiggins, 1960; Sweatman and Henshall, 1962; Gemmell, 1970a), *E. granulosus* (Dew, 1928; Dévé, 1949; Yamashita *et al.*, 1961; Lupaşcu and Panaitesco, 1968) and *E. multilocularis* (Rausch, 1954; Ohbayashi *et al.*, 1971) showed that considerable variation exists between the size of individual organisms in the normal intermediate host.

One of the more important problems in defining the infective pattern quantitatively, concerns the detection of small lesions comprising parasitic remnants and the reaction by the host to them. Gibson (1959) drew attention to the difficulty in identifying parasitic remnants of *T. saginata*. This is also the case in distinguishing between degenerated *T. hydatigena* and *E. granulosus* in the liver (Gemmell, 1968). The larvae of *T. hydatigena* can be more readily detected on the surface of liver than those of *T. ovis* buried deep in the muscles of sheep. In some instances, many remnants may have disappeared before autopsy. In addition, survival rates may depend on the time interval between infection and autopsy.

A further problem concerns the assessment of the significance of the ratios of dead and viable organisms. The difference between the total number of cysts in test and control animals has been used as an index and termed "early" (Campbell, 1936, 1938a,b,c) or "pre-encystment" (Gemmell and Soulsby, 1968) immunity. Similarly, the terms "late" or "post-encystment" immunity have been used to describe proportional differences in the survival rates between immune and control animals. These indices are useful descriptive terms for epidemiological studies but are probably too imprecise for describing the complex events in naturally acquired immunity. Despite these limitations, there is obvious merit in any experimental approach to the host–parasite

relationship in distinguishing between dead and viable cysts and in determining the infective patterns from total as well as from surviving cysts. Because of the non-normality of the distributions of these metacestode populations, we have compared treated groups of animals with each other and with controls by using an analysis of variance and confirming the results of these analyses by a randomization test. In other cases, randomization tests on their own have been used to test for differences between appropriate groups. Differences in the survival rates of larval forms between test and control animals have been determined by the Hierarchical $\chi^2$ test (Fisher, 1954).

## IV. Innate Resistance and the Infective Pattern of Larval Cestodes

Crofton (1971a,b) fitted the infective pattern of an acanthocephalan in an amphipod by a negative binomial and suggested that in that ecosystem, aggregation permitted the maintenance of an equilibrium between the parasite and host; whereby the parasite regulated the host population and was in turn regulated by it.

Little is known of the infective pattern of larval cestodes in sheep, cattle and pigs or the extent to which such factors as age, sex and breed of the host, nutritional factors and grazing pattern modify it.

The homogeneity of an animal population's susceptibility to infection can be tested by the binomial index of dispersion (Fisher, 1954):

$$\chi^2_{n-1} = \frac{\sum_{i=1}^{n} (x - \bar{x})^2}{np(1-p)}$$

where $n$ = the number of animals, $p$ = the proportion of cysts established or surviving from a given number of eggs, and $x$ is the number of cysts observed in $i^{\text{th}}$ animal.

It is essential to protect experimental animals from accidental infections. The methods described in Gemmell *et al.* (1969) have been used in an attempt to obtain uniform batches of colostrum-fed and colostrum-deprived lambs for almost all the experiments with the sheep metacestodes analysed in this review. The general conditions under which the lambs were reared are illustrated in Fig. 1.

Eggs of *T. hydatigena*, *T. ovis* and *E. granulosus* were fed to artificially reared lambs at 3 to 6 months of age (Gemmell, 1964a, 1965a, 1966, 1969a, b). Cyst counts from feeding 10, 100, 1000 or 2500 eggs showed that the binomial index of dispersion increased as the number of eggs fed was increased (Table I). Although the estimates were not obtained from experiments specifically designed to define the infective pattern, they are consistent with a wide departure from a binomial distribution and with the hypothesis that in any host population there is a threshold of innate resistance to larval cestodes and this varies from one animal to the next. A similar divergence from a binomial distribution can be detected for the superimposed *T. saginata* infection in calves described in Mango and Mango (1972).

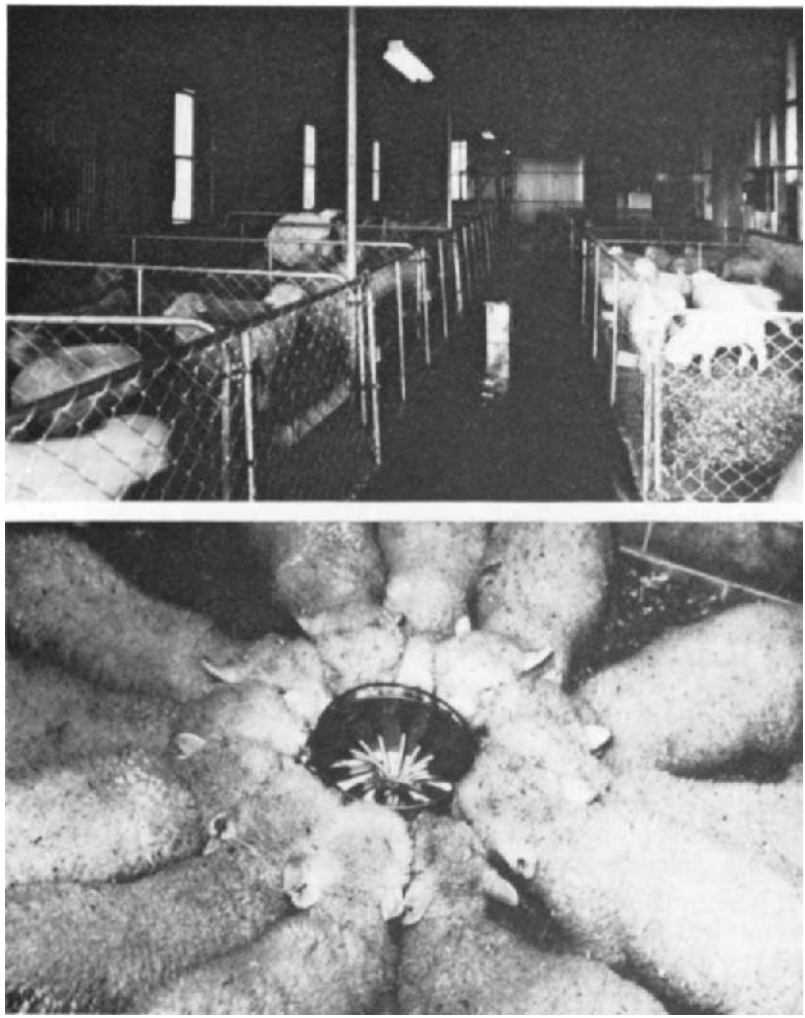


FIG. 1. Lambs artificially reared in a tapeworm-free unit for epidemiological studies on hydatidosis and cysticercosis.

## V. INTRINSIC FACTORS MODIFYING THE INFECTIVE PATTERN IN THE HOMOLOGOUS SITUATION

It is necessary to summarize some potential interactions that may help to explain the importance of antibody and cell-mediated immunity (CMI) in limiting larval cestode populations. Sheep and cattle have not yet been used for basic studies on these aspects. Most studies have been undertaken with *T.*

TABLE I

*Variation in larval cyst counts of* Taenia hydatigena, T. ovis *and* Echinococcus granulosus *from a specified number of eggs in a single dose*

| | *T. hydatigena* | | | | *T. ovis* | | | | *E. granulosus* |
|---|---|---|---|---|---|---|---|---|---|
| Eggs fed | 10[e] | 100[e] | 1000[e] | 2500[a,d] | 10[e] | 100[e] | 1000[e] | 2300[b,c,d] | 2500[c] |
| | | | | TOTAL CYSTS | | | | | |
| Propn infected | 5/6 | 6/6 | 10/10 | 20/20 | 6/9 | 7/8 | 7/8 | 20/20 | 9/9 |
| Range | 0–5 | 6–38 | 6–122 | 97–500 | 0–6 | 0–6 | 0–140 | 31–243 | 19–154 |
| Mean ± SD | 2·5 ± 1·5 | 21·6 ± 11·8 | 64·3 ± 37·6 | 306·1 ± 123·7 | 1·4 ± 1·8 | 3·5 ± 2·0 | 71·8 ± 45·7 | 134·6 ± 53·6 | 69·7 ± 40·5 |
| Binomial index of dispersion | 12·0 | 833*** | 23485*** | 174324*** | 27** | 118*** | 31338*** | 56437*** | 60521*** |
| | | | | VIABLE CYSTS | | | | | |
| Propn infected | 3/6 | 5/6 | 10/10 | 20/20 | 1/9 | 2/8 | 2/8 | 16/20 | 9/9 |
| Range | 0–4 | 0–15 | 1–93 | 1–307 | 0–1 | 0–4 | 0–21 | 0–55 | 1–100 |
| Mean ± SD | 1·0 ± 1·4 | 8·5 ± 5·9 | 45·0 ± 29·4 | 142·4 ± 111·9 | 0·1 | 0·8 ± 1·4 | 3·0 ± 6·9 | 14·5 ± 19·9 | 28·4 ± 29·4 |
| Binomial index of dispersion | 22*** | 448*** | 201113*** | 233110*** | — | 247*** | 15918*** | 68676*** | 76962*** |

Level of significance of departure from Binomial distribution: ***$P < 0.001$ **$P < 0.01$.

(a) Gemmell (1964); (b) Gemmell (1965a); (c) Gemmell (1966); (d) Gemmell (1969); (e) Gemmell (1969b).

*pisiformis* and *T. taeniaeformis* in rabbits and rats respectively. Silverman (1955a) and Heath (1970) demonstrated that serum from immune rabbits killed activated embryos of *T. pisiformis in vitro*. There was no correlation between antibody demonstrated by conventional tests and lethal effects on the embryo. Rickard and Outteridge (1974), using *T. pisiformis*, measured "lethal" antibody by titration and absorption methods with activated embryos and CMI by delayed hypersensitivity skin reactions and *in vitro* lymphocyte transformation. They suggested that antibody may kill the early developmental stages, whereas CMI may be important later.

Taking this a step further, Rickard (1974) proposed the hypothesis that when embryos enter an immunologically "naïve" animal, they develop rapidly and reach an antibody-resistant stage before specific immunoglobulin is produced in sufficient amounts. These organisms then become coated with other specific antibody which acts as blocking antibody, interfering with the more slowly developing CMI. Rickard and Katiyar (unpublished) suggested that antigens stimulating immunity against the establishment of *T. pisiformis* and those provoking CMI may be different. In "primed" animals, however, the embryos are rapidly killed before reaching the resistant stage. This is essentially the "trap and mask" theory proposed for schistosomiasis by Smithers *et al.* (1969) and for *E. granulosus* by de Rycke and Pennoit-de Cooman (1973) and by Varela-Díaz and Coltorti (1973). Specific antibodies to various antigens should determine the total number of organisms that start development. These represent the early and late immunity suggested by Campbell (1936, 1938a,b,c). Cell-mediated immunity should determine the number of survivors.

This attractive hypothesis emphasizes temporal effects and the plurality of the immune responses. It permits the view that some of the early lethal effects may operate in the gut in "primed" animals (Leonard and Leonard, 1941; Froyd and Round, 1959, 1960; Gemmell, 1962; Heath, 1970, 1971, 1973a) as well as in their tissues (Gemmell and Macnamara, 1972). Some effects operating later, may involve antigens on the cyst wall (Varela-Díaz *et al.*, 1972).

Almost certainly, some of the early immune effects involve excretory/secretory (ES) antigens. Whether these include the secretions from the penetration glands described by Silverman (1955a) and Silverman and Maneely (1955) remains to be determined. Heath and Pavloff (1974), using *T. taeniaeformis*, confirmed Campbell's (1938c) observations that at least two immune reactions can be distinguished by serum transfer studies. They considered that a susceptible phase of development exists between 5 and 11 days after invasion. Death at this phase results in macroscopic lesions. Death of larvae prior to this results in no observable lesions. Heath and Pavloff suggested that early immunity is induced by antigens shared by oncosphere and cyst tissue and late immunity is stimulated by exo-antigens specific to the developing larvae. Heath (1973b) reported that larvae of *T. pisiformis* grown *in vitro* for 15 days conferred strong resistance to a challenge infection, but protective immunity decreased after this stage and implanted mature cysts provided no protection against reinfection.

The culture of oncospheres *in vitro* (Heath and Smyth, 1970; Heath and Elsdon-Dew, 1972; Heath, 1973c) and in *in vivo* millipore filter diffusion chambers (Rickard and Bell, 1971a,b,c) and in embryonated hen eggs (Parmeter and Gemmell, 1974) should assist in defining more precisely the sequence of events that leads to the early and late deaths as well as that which gives rise to the long-term survival of the larval forms in an otherwise immune host.

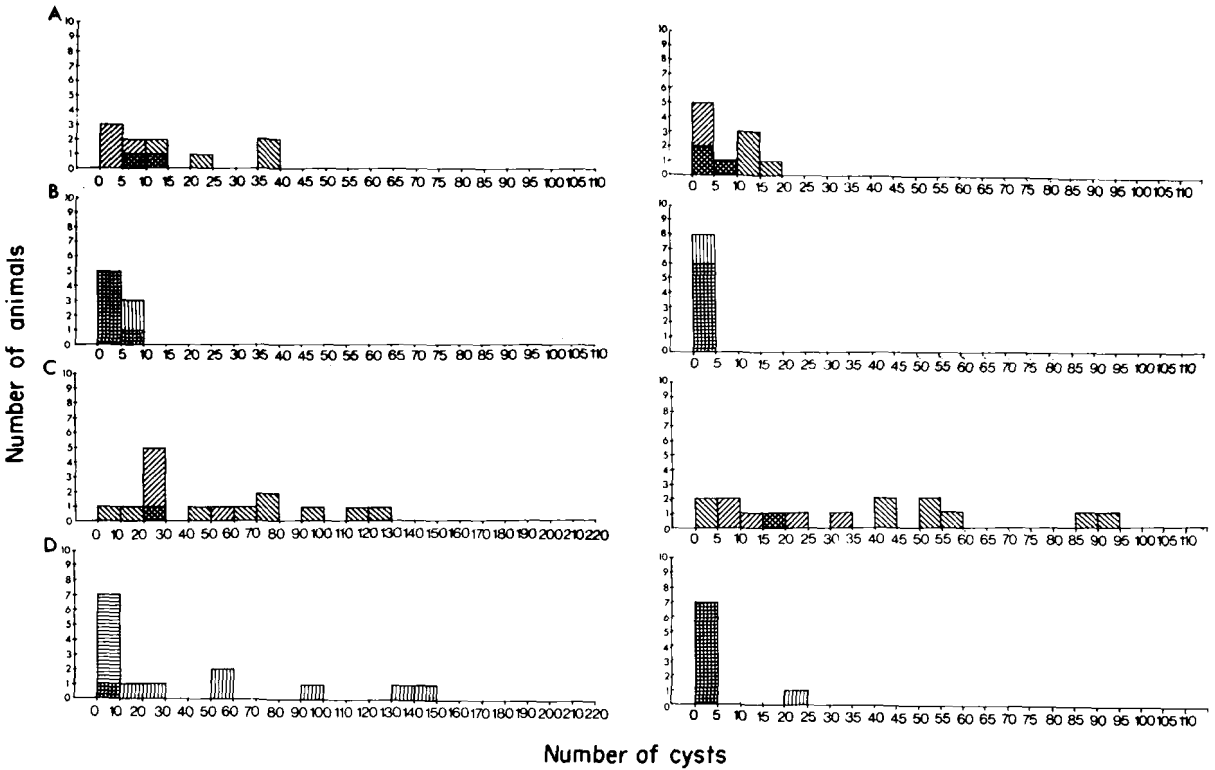
The ability of parasites to survive in tissues of immune animals has become a central issue in immunoparasitology (Varela-Díaz and Coltorti, 1973). Special mechanisms should exist to regulate growth and asexual proliferation in tissue-proliferating forms such as *Echinococcus*. In some cases, cysts survive but are sterile. In others, massive proliferation of germinal tissue and/or protoscoleces proceeds unchecked. In a series of studies it has been shown that IgG penetrates into and through the laminated membrane but it appears to act as a barrier to cells. The germinal membrane plays a regulatory role for macromolecules. In addition, the healthy hydatid cyst may only produce a low level of antigenic stimulation, perhaps insufficient to induce a host response adverse to survival (Varela-Díaz and Coltorti, 1972, 1973; Coltorti and Varela-Díaz, 1972, 1974; Varela-Díaz *et al.*, 1974). Rau and Tanner (1972, 1973) and Baron *et al.* (1974a,b), working on the population regulation of *E. multilocularis* in cotton rats, suggested that in secondary echinococcosis, an established cyst may inhibit the growth of its peripheral conglomerates. They suggested that killing is a function of CMI and that adherence of cells may be mediated by antibody that furnishes recognition sites on the protoscolex. They also suggested that complement may be inactivated by calcareous corpuscles, thereby preventing fixation of complement by the antibody. Clearly there is some control but the precise mechanisms remain to be elucidated.

### A. ACQUIRED IMMUNITY

Some of the earliest experiments demonstrating acquired immunity to metazoan parasites in large animals were carried out with *T. saginata* in cattle (Penfold *et al.*, 1936a; Penfold and Penfold, 1937). Urquhart (1958, 1961) and Froyd (1964a) extended knowledge particularly on the acquisition of immunity to *T. saginata* from infections established early in postnatal life, thereby demonstrating the difficulty of working with the experimental system in endemic regions. Gallie and Sewell (1972) reproduced these events experimentally and confirmed that calves that have acquired resistance to reinfection continued to harbour cysts from an earlier infection. Sweatman (1957) demonstrated that sheep acquired strong resistance to *T. hydatigena* within 7 weeks of feeding eggs. It was also shown that partial resistance to *E. granulosus* could be detected after 9 months (Sweatman *et al.*, 1963). These important studies were concerned primarily with demonstrating the rejection of a superimposed infection.

#### *Size and frequency of infections*

The size and frequency of the egg doses ingested before the onset of resistance should determine *inter alia* the infective pattern and survival rates of the cystic


A
B
C
D
Number of animals
Number of cysts
Left, total cysts; right, viable cysts. T. hydatigena treated, control. T. ovis treated, control.
E. granulosus treated, control.

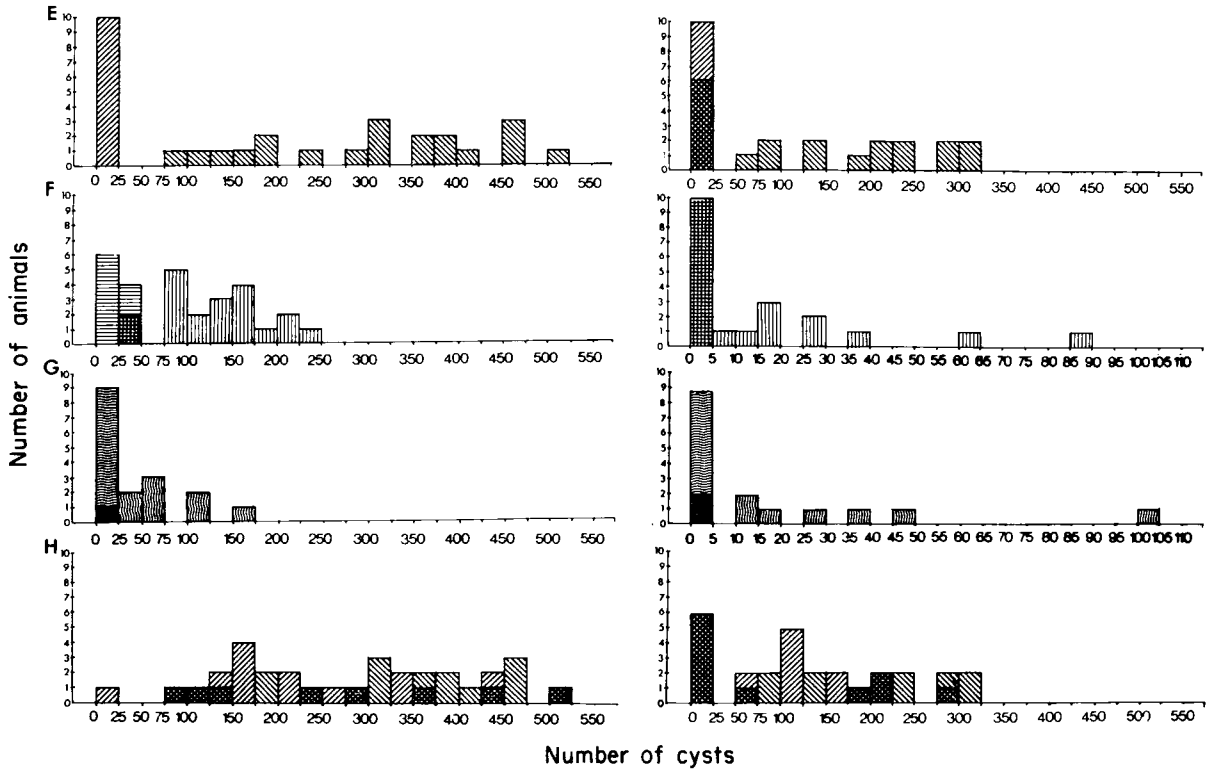



FIG. 2. Class frequency distribution of larval cestodes in sheep. A,B: Ten doses of 10 eggs over 9 weeks and a single dose of 100 eggs (control). C,D: Ten doses of 100 eggs over 9 weeks and a single dose of 1000 eggs (control). E,F,G: Single dose of 2500 eggs in sheep immunized with homologous activated embryos and in non-immunized sheep (control). H: Single dose of 2500 eggs in sheep immunized with killed embryos and in non-immunized sheep (control).

forms. To test the importance of these parameters, lambs were fed with 100 or 1000 eggs of *T. hydatigena* or *T. ovis* in ten divided doses at 7-day intervals over a period of 9 weeks (Gemmell, 1969b). The cyst counts at autopsy were compared with those obtained by feeding the same number of eggs on a single occasion. Class frequency data are illustrated in Fig. 2.

Significantly ($P < 0{\cdot}05$) more cysts of *T. hydatigena* developed and survived from 1000 than from 100 eggs fed on a single occasion. Significantly more organisms developed and survived from 100 eggs fed in a single dose than from 100 eggs fed in divided doses. However, when 1000 eggs were given in a single dose, the number of cysts did not differ significantly from that when 1000 eggs were given in divided doses, although the number of viable cysts was marginally more in the single dose group. In the case of *T. ovis* high cyst counts with many survivors were observed only in the lambs receiving 1000 eggs on a single occasion.

While the cysts that developed could not be identified with a particular dose of eggs, the results are consistent with the hypothesis that the size of the initial egg dose affects the total number of lesions and the surviving cysts. The frequency of infection assumes importance only during the limited period between the ingestion of the first eggs and the onset of resistance.

### B. INDUCED IMMUNITY

When small doses of eggs are repeatedly ingested, immune responses may not be identical to those when large number of eggs are injected on a single occasion. The parenteral injection of organisms should give a primary exposure to the lymphatic–reticuloendothelial system and simulate some of the immunological events induced by feeding eggs. Such manipulations allow a more accurate evaluation of immunity in regulating larval cestode populations than can be achieved by feeding both the immunizing and challenge dose of eggs and attempting to separate the cysts established by each procedure.

Some of the earliest immunization studies using activated embryos injected into ectopic sites were those of Froyd and Round (1959, 1960). Activated embryos of *T. saginata* were injected into the muscles of cattle that had been naturally infected and had acquired a degree of resistance to superinfection. The injected organisms developed and it was inferred that the injection had by-passed an intestinal phase of immunity described by Leonard and Leonard (1941).

Hatching of embryos of *E. granulosus* may occur in tissues (Batham, 1957; Borrie *et al.*, 1965). When eggs of *T. hydatigena* and *T. ovis* were injected into susceptible sheep, embryos rarely developed. When embryos were artificially hatched and activated, however, they were then capable of developing into cysts at the site of injection and in the lymph nodes draining the site (Gemmell, 1969c).

In the trials described below viable or killed eggs or activated embryos of *T. hydatigena*, *T. ovis* or *E. granulosus* were injected into the muscles of lambs (Gemmell, 1964a, 1965a, 1966). The animals were dosed 3 months later with 2500 homologous eggs. Autopsy was carried out 3 months after the challenge

infection was administered, with the exception of *E. granulosus* which required 30 months for adequate development. Class frequency data are illustrated in Fig. 2.

1. *Immunity induced by viable organisms*

Strong immunity was induced by the parenteral injection of artificially activated embryos. For *T. hydatigena*, *T. ovis* and *E. granulosus* there was a highly significant ($P < 0{\cdot}01$) reduction in the total cyst counts and a virtual absence of viable cysts from the challenge infection. Similar results were obtained when viable eggs were used to immunize lambs.

2. *Immunity induced by killed organisms*

In contrast to viable organisms, no significant reduction in the total or viable cyst counts was observed when the eggs of *T. hydatigena* used as the primary inoculum were killed by deep freezing at $-70°C$ (Gemmell, 1964a) or by sonication (Gemmell, 1969c). Similar results were obtained using *T. ovis*. *Echinococcus* was not tested.

In the experiments using viable organisms, quantitation of the primary inoculum was considered not to be critical. This may not be the case with killed organisms. The failure to protect may have been due either to the destruction of the immunogens by the techniques used to kill the embryos or to quantitative effects. Hydatid cyst fluid does not induce immunity to *E. granulosus* in sheep (Moya and Blood, 1964). These results are consistent with the hypothesis that protection-inducing antigens may be absent or may be present in only very small quantities in such material.

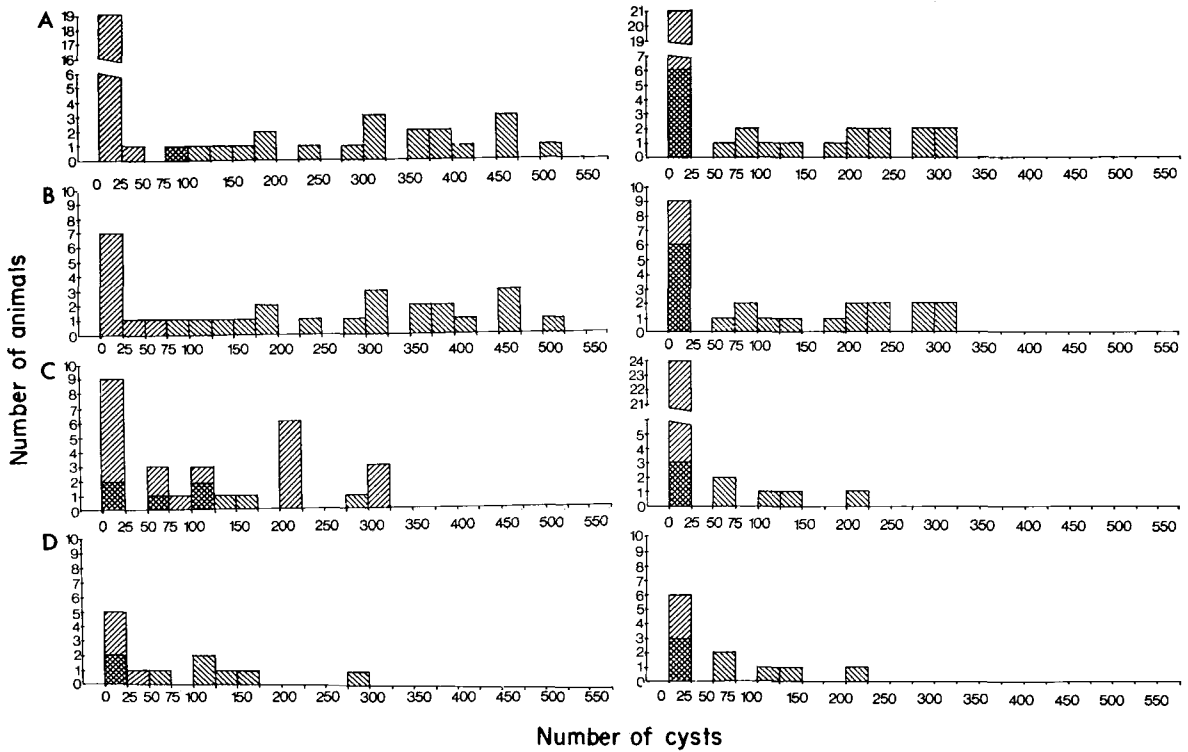
3. *Immunity in the absence of larval development*

Evidence is required to determine whether cyst survival is necessary for the development of strong immunity. Lambs were injected with the embryos of *T. hydatigena* modified by various physical or chemical procedures (Gemmell, 1969c). They were challenged with the eggs of *T. hydatigena* by the oral route. In about half the animals no cysts developed from the primary inoculum at the site of injection. There was a highly significant reduction ($P < 0{\cdot}01$) in both the total and viable cyst counts of the challenge infection. The immunity induced was virtually absolute, irrespective of the survival or death of the primary inoculum. Class frequency data are illustrated in Fig. 3. Similar results were obtained for *T. ovis* (data not shown).

It seems likely that short-term survival of embryos, but not necessarily complete reorganization into cysts, may be required for the induction of a strong immunity.

4. *Excretory/Secretory (ES) antigens*

Immunogens obtained from the *in vitro* culture of embryos or from organisms implanted in the host in millipore filter diffusion chambers for specific periods, have been used to immunize sheep against *T. ovis* by Rickard and Bell (1971a,




Left, total cysts; right, viable cysts. *T. hydatigena* treated, control.

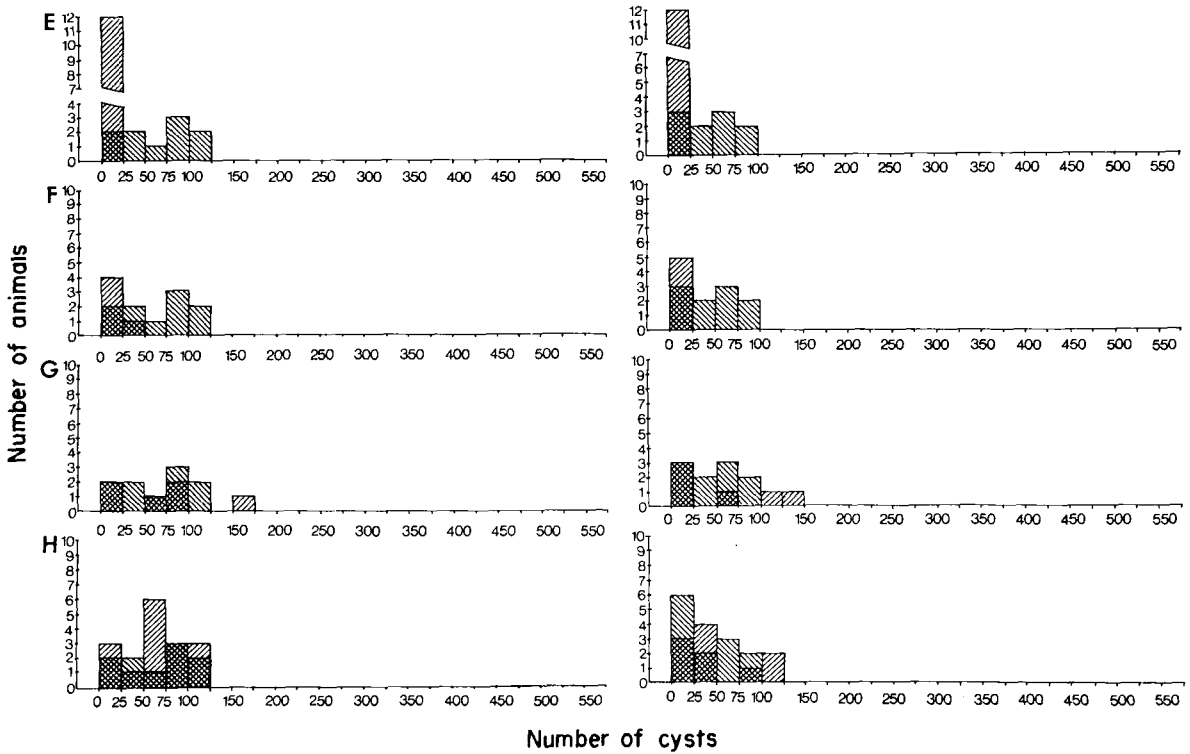



FIG. 3. Class frequency distribution of larval cestodes in sheep. A,B: 2500 eggs in sheep immunized at 3 months of age with homologous embryos that (A) developed into cysts, (B) did not develop into cysts at the site of injection and in non-immunized sheep (control). C,D: 1000 eggs in sheep immunized with homologous embryos at (C) 1–4 weeks of age, (D) 8 weeks of age and in non-immunized sheep (control). E,F,G,H: 100 eggs in sheep immunized with homologous embryos (E) 2–3 weeks before, (F) 1 week before, (G) simultaneously with (H) 2–3 weeks after challenge and in non-immunized sheep (control).

b,c). Absolute immunity to *T. ovis* was induced in lambs from material collected from embryos cultured for 8 days and injected with adjuvant or from embryos implanted in the host in diffusion chambers and removed after 7 days. Since there was complete rejection of the challenge infection, it could not be determined whether all ES antigens were produced during the culture period. Nevertheless, the results are consistent with the hypothesis that important ES antigens are expressed by embryos within 7 days of the initiation of reorganization. Further work is required to define the morphological changes associated with the expression of the various antigens.

### C. IMMUNITY IN YOUNG ANIMALS

Calves naturally infected shortly after birth gradually acquire resistance to *T. saginata* (Urquhart, 1961). However, immunization at one week of age was unsuccessful (Froyd, 1961). Furthermore, calves less than 4 weeks old remained susceptible to reinfection, but by 4 months were strongly resistant to further challenge (Froyd, 1964a). Calves given only a single dose of eggs shortly after birth were not resistant to reinfection at 12 months of age, whereas those repeatedly fed with eggs during the 12-month period were strongly resistant (Gallie and Sewell, 1972). Gallie and Sewell (1974a,b) recorded that a single dose of eggs did not give absolute protection to 3-month-old calves challenged 10 months later. Soulsby (1963) indicated that calves infected at 4–6 months were resistant to challenge 9 months later.

Evidence is available to demonstrate the changing ability of very young animals to acquire resistance to the various larval tapeworms. Lambs that had received colostrum were inoculated with embryos of *T. hydatigena* at 1, 2, 4 or 8 weeks of age. They were then challenged with 2500 eggs of *T. hydatigena* and subsequently autopsied (Gemmell *et al.*, 1968a). The data for the ages 1, 2 and 4 weeks did not differ and have been combined and are illustrated in Fig. 3. There was a significant difference ($P < 0{\cdot}01$) in the total cyst counts only between the lambs vaccinated at 8 weeks and the controls. There was, however, a significant difference ($P < 0{\cdot}01$) in the viable cyst counts between all groups and the control. Indeed, cysts failed to survive in almost all animals irrespective of the age at the time of vaccination. Although the antibody content of the colostrum was unknown, these results are consistent with the hypothesis that several weeks may elapse before lambs become capable of responding to the full range of larval antigens.

#### *Tolerance*

Soulsby (1963, 1965) detected a degree of serological unresponsiveness possibly associated with tolerance to *T. saginata* when calves were infected shortly after birth. Urquhart (1961, 1970) discussed the possibility that *T. saginata* infections in very young cattle might induce tolerance. Gemmell and Macnamara (1972) considered that due to various factors such as a period of relative immunological incompetence of calves (Smith and Ingram, 1965) and variable effects that might occur from different egg doses, there was little evidence to support the concept of tolerance in aiding survival in larval cestode

infections. Gallie and Sewell (1972, 1974b) showed that although infected calves do not develop a strong serological response, an early infection does not interfere with their ability to develop resistance to reinfection with *T. saginata*.

### D. TIME INTERVAL FOR THE INDUCTION OF IMMUNITY

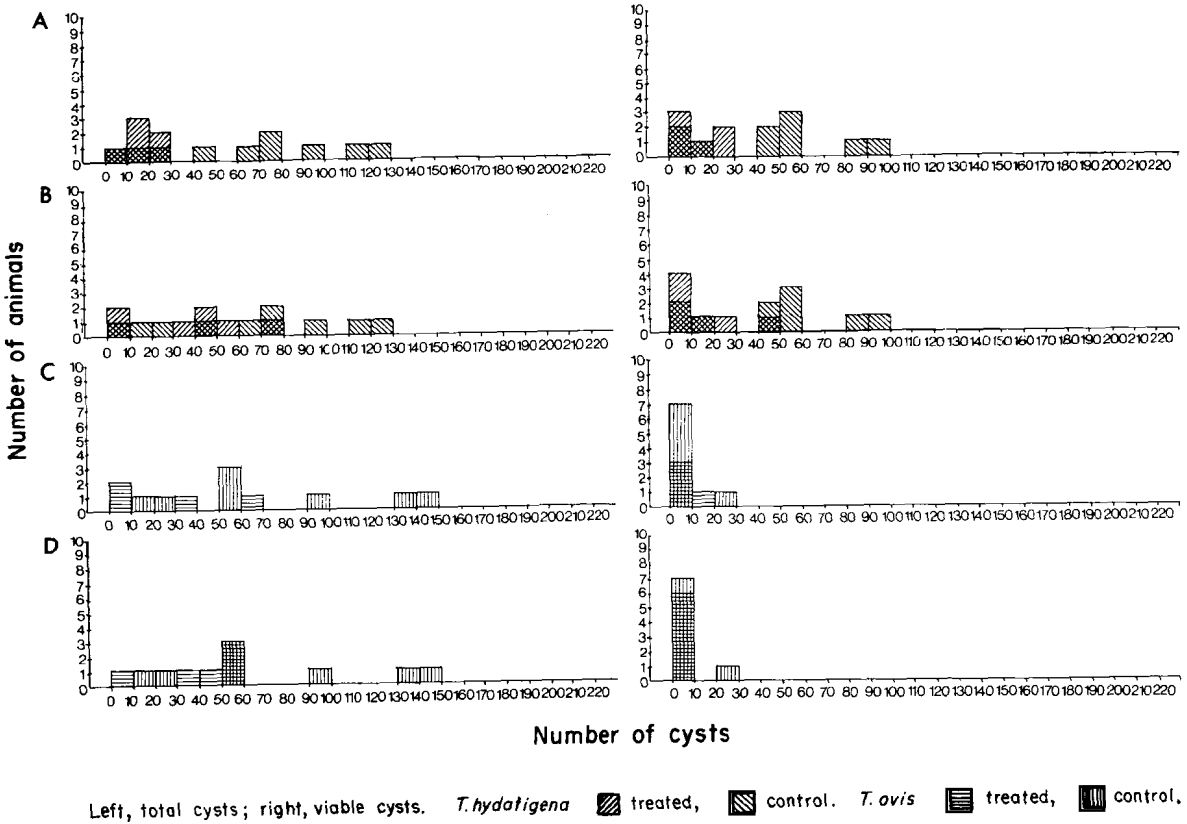
Groups of 3-month-old lambs were inoculated with activated embryos of *T. hydatigena* injected by the intramuscular route (Gemmell *et al.*, 1968b). Some animals were given 1000 eggs *per os* up to 3 weeks before and others were challenged simultaneously or up to 3 weeks after inoculation. Class frequency data are recorded in Fig. 3. There was a significant ($P < 0{\cdot}01$) reduction in total and viable cyst counts in the lambs inoculated one or more weeks before the challenge infection. Indeed, partial and absolute immunity was demonstrated at 1 and 2 weeks respectively. No significant lethal effects were detectable either against total numbers or survival rates when the inoculation was given when or after the eggs had been fed. In this case, the initial oral infection induced an immunity that prevented the inoculum from developing. The results are consistent with the hypothesis that during the interval between the initial infection and the onset of resistance to superinfection, a period of up to 2 weeks, some cysts may still develop and survive from ingested eggs.

### E. DURATION OF IMMUNITY

The survival of cysts of *T. saginata* in cattle has been variously described as only a few months or several years (Penfold, 1937; Penfold and Penfold, 1937; Penfold *et al.*, 1936a; Jepsen and Roth, 1952; Peel, 1953; McIntosh and Miller, 1960; Friedrich, 1961; Soulsby, 1963; Dewhirst *et al.*, 1963; Froyd, 1964b; Urquhart and Brocklesby, 1965; Koudela, 1967; Van den Heever, 1967; Vegors and Lucker, 1971; Nadzhavof, 1971; Leikina *et al.*, 1972). Although it is uncertain from the data presented, Leikina *et al.* (1964) suggested that immune effects on established cysts can be induced by a superimposed infection. The importance of continuing infections in modifying the infective pattern of established cysts has yet to be determined. Abuladze *et al.* (1973) reported that the viability of the cysticerci of *T. saginata* is inversely proportional to the numbers of superinfections. Soulé *et al.* (1971, 1972) also observed that antibody levels declined after infection and that subsequent infections may modify the infective pattern. Reciprocal effects between embryos and cysticerci of *T. saginata* have been reported (Kosminkov, 1973). We have been unable to find any definitive experiments reporting changes in survival rates of *T. saginata* or *T. solium* in immunologically competent hosts. This is also the case for the sheep metacestodes.

Similarly, a search of the literature has failed to reveal experiments specifically demonstrating the induction of immunity with or without the persistence of the original organisms followed by a reduction in its effectiveness over time.

Circumstantial evidence suggests that immunity associated with early death


A
B
C
D
Number of animals
Number of cysts
Left, total cysts; right, viable cysts. T. hydatigena treated, control. T. ovis treated, control.

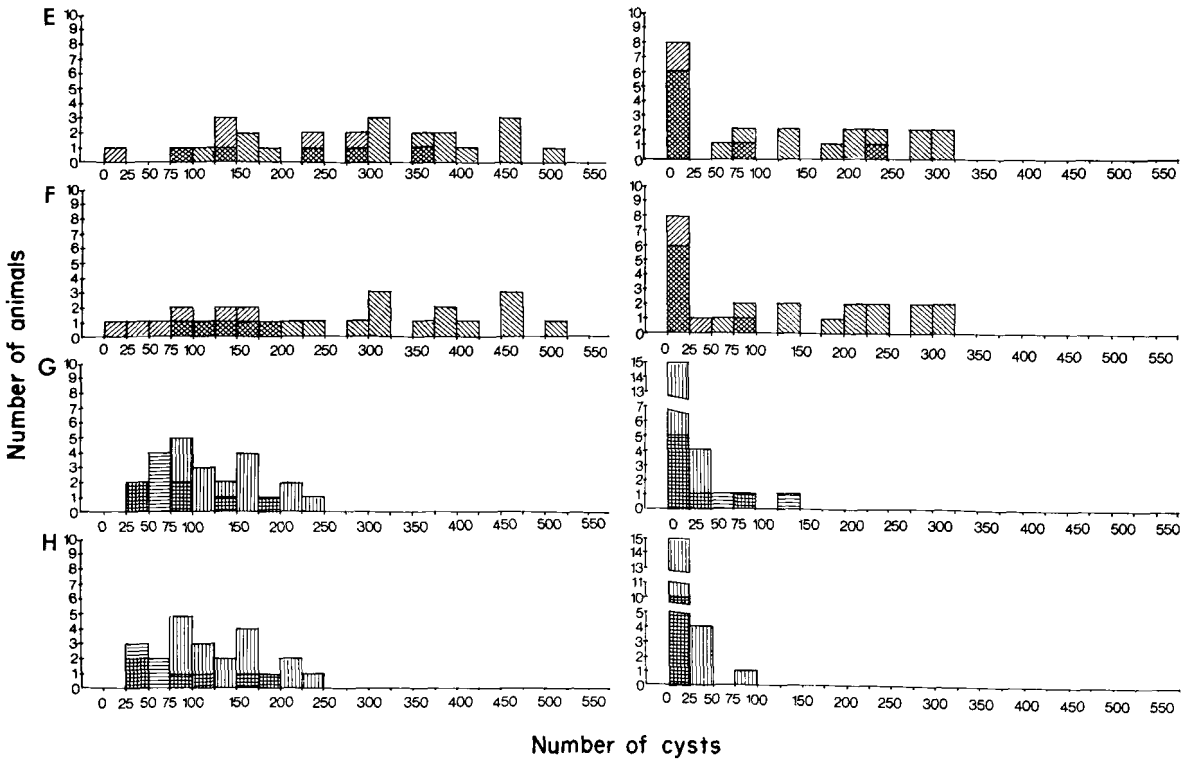



FIG. 4. Class frequency distribution of larval cestodes in sheep. A,B: 1000 eggs of *T. hydatigena* in sheep previously fed with 10 divided doses of (A) 100 eggs, (B) 1000 eggs of *T. ovis* and in previously untreated sheep (control). C,D: 1000 eggs of *T. ovis* in sheep previously fed with 10 divided doses of (C) 100 eggs, (D) 1000 eggs of *T. hydatigena* and in previously untreated sheep (control). E,F: 2500 eggs of *T. hydatigena* in sheep previously fed (E) before (F) simultaneously with 2500 eggs of *T. ovis* and in previously untreated sheep (control). G,H: 2500 eggs of *T. ovis* in sheep previously fed (G) before (H) simultaneously with 2500 eggs of *T. hydatigena* and in previously untreated sheep (control).

of invading organisms may wane in the absence of continuing ingestion of eggs. The duration of immunity may differ with different antigens and immune mechanisms. For example, if the immunity associated with "early" deaths waned but that involving "late" deaths was retained, organisms from a challenge infection might develop and then die. The animal would then be heavily loaded with dead cysts from the superimposed infection.

## VI. Factors Modifying the Infective Pattern in Heterologous Systems

In cattle, *T. saginata* and *E. granulosus* are not mutually exclusive (Froyd, 1960). Surveys have also shown that *T. hydatigena*, *T. ovis* and *E. granulosus* may occur together in the same sheep (Gemmell, 1961b,c,d, 1968). Rabbit metacestodes such as *T. pisiformis* frequently occur in the same ecosystem as the sheep metacestodes. The importance of interspecific competition by larvae in modifying the infective pattern is difficult to assess from survey data and this subject has to be approached with caution.

Two systems have been studied. In the first, estimates have been made of the extent to which *T. hydatigena*, *T. ovis* and *E. granulosus* can co-exist in sheep. In the second, evidence is examined for cross-protective immune responses between these sheep metacestodes and *T. pisiformis* in their respective intermediate hosts.

### A. Species Parasitizing Similar Hosts

#### 1. *Aquired resistance*

Two egg-feeding experiments provide evidence of the extent to which *T. hydatigena* and *T. ovis* may co-exist in the same animal. Class frequency data are illustrated in Fig. 4. In the first trial, lambs were "primed" with one species by feeding 100 or 1000 eggs in divided doses over a period of 9 weeks. They were the challenged with 1000 eggs of the heterologous species (Gemmell, 1969b). With the exception of the total cyst counts in the group first fed with 100 *T. ovis* and upon which *T. hydatigena* was superimposed, the total and viable cyst counts of the superimposed species did not differ significantly from the controls fed with one species only. The superimposed species was generally able to invade, reorganize and survive.

In the second trial, lambs were fed with 2500 eggs of *T. hydatigena* and 2500 eggs of *T. ovis* together or separated by an interval of 3 months (Varela-Díaz *et al.*, 1972). In the case of *T. hydatigena* when *T. ovis* was given beforehand or simultaneously, there was a significant reduction ($P < 0{\cdot}05$) in both the total and viable cyst counts and in their proportions (Fig. 4). In the case of *T. ovis*, the only difference was a greater proportion surviving in the lambs that had previously been fed with *T. hydatigena*. These egg-feeding trials indicate that taeniid species may survive together in the same host, but interactions may be neutral, harmful or possibly beneficial to the parasite. No evidence was found suggesting that interspecific effects were as important in modifying the infective pattern as species-specific regulation by the host.

2. *Induced immunity*

In a series of reciprocal experiments, 3-month-old lambs were injected with activated embryos of *T. hydatigena* or *T. ovis* (Gemmell, 1964a,b, 1965a, 1966, 1967). They were challenged with 2500 eggs of the heterologous species or with *E. granulosus* 3 months later. The lambs were autopsied 3 months after the challenge, except for the last named which required 30 months. Class frequency data are illustrated in Fig. 5.

In all these studies, there was a significant reduction ($P < 0{\cdot}05$) in the total cyst counts of *T. hydatigena* and *T. ovis* but none for *E. granulosus*. There was a striking reduction in the number and proportion of cysts of *T. hydatigena* that survived in the sheep immunized with *T. ovis*. This was not observed for *T. ovis* in sheep immunized with *T. hydatigena*, but was evident for *E. granulosus* in both systems. Indeed, hydatid cysts failed to survive in the majority of animals even when more than 100 larvae commenced development.

These cross-protective effects on survival were evident when the primary inoculum consisted of embryos. When it consisted of eggs, the only effect on *T. hydatigena* and *T. ovis* was a reduction in the total cyst counts for the heterologous species (data not illustrated). The hydatid organisms were not affected by injecting heterologous eggs. These results suggest that some similar antigens occur in viable embryos of taeniids that parasitize sheep.

3. *Excretory/Secretory (ES) antigens*

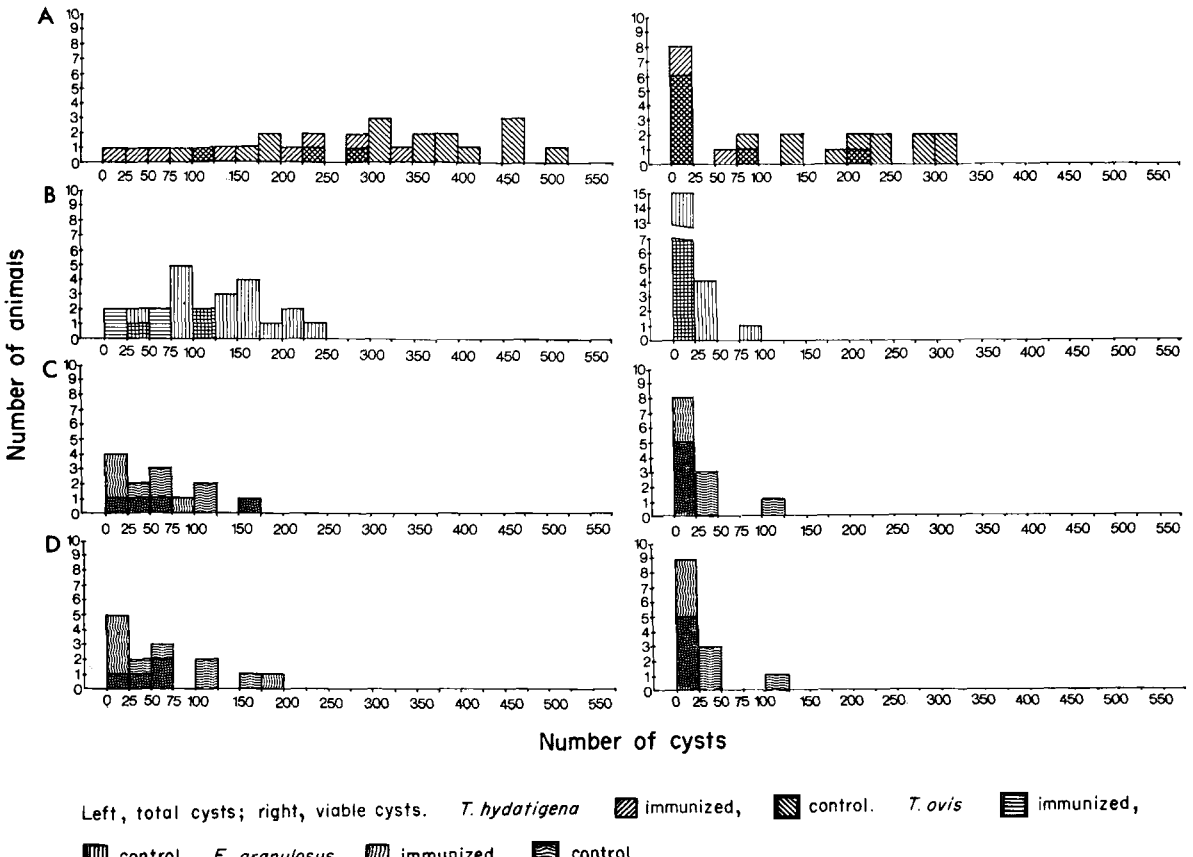
Rickard and Bell (1971c) reported that secretions collected over an 8-day period from cultured *T. hydatigena* induced immunity against *T. ovis* when injected with Freund's Complete Adjuvant into 6-week-old lambs. Further work is required to identify the stages during the reorganization of the larval forms when species-specific and cross-protective metabolic and somatic antigens are predominately expressed.

## B. SPECIES PARASITIZING DIFFERENT HOSTS

Some sheep metacestodes such as *T. hydatigena* and *E. granulosus* co-exist naturally with *T. saginata* in the same cattle beast. A preliminary report has indicated that a partial immunity can be induced against *T. saginata* by an injection of embryos of *T. hydatigena* (Wikerhauser *et al.*, 1971). Little is known of the interspecific effects of *T. solium* and other taeniids. Information is, however, available on reciprocal effects between *T. pisiformis* and some sheep metacestodes.

1. *Acquired immunity*

Natural infections with *T. pisiformis* in sheep have been observed (Grintescu, 1968). It may develop occasionally following the intravenous injection of embryos into sheep (Gemmell, 1962). However, the sheep is an abnormal host. Reciprocal egg-feeding experiments have so far only been studied in rabbits. Ermalova *et al.* (1969) showed that although *T. hydatigena* did not survive for any length of time in the rabbit, some immunity was acquired to *T. pisiformis*


A
B
C
D
Number of animals
0 25 50 75 100 150 200 250 300 350 400 450 500 550
Number of cysts
Left, total cysts; right, viable cysts. T. hydatigena immunized, control. T. ovis immunized, control. E. granulosus immunized, control.

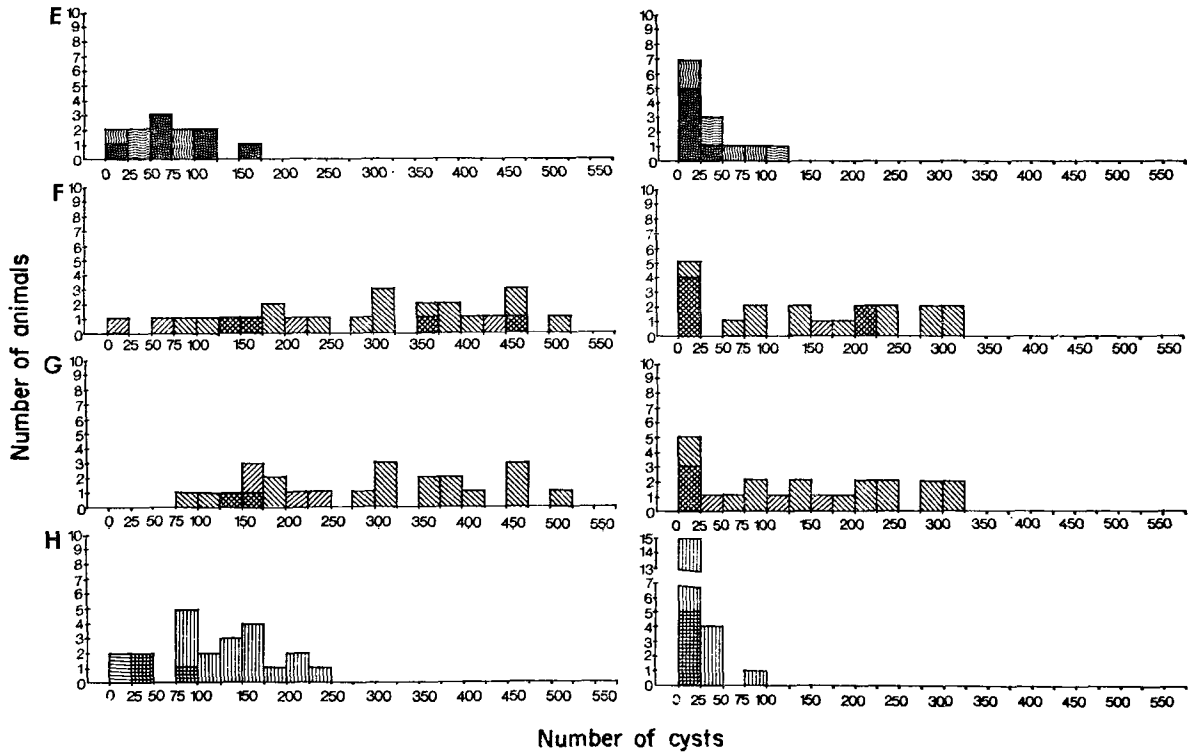



FIG. 5. Class frequency distribution of larval cestodes in sheep. A: 2500 eggs of *T. hydatigena* in sheep immunized with viable embryos of *T. ovis* and in untreated sheep (control). B: 2500 eggs of *T. ovis* in sheep immunized with viable embryos of *T. hydatigena* and in untreated sheep (control). C,D,E: 2500 eggs of *E. granulosus* in sheep immunized with viable embryos of (C) *T. hydatigena*, (D) *T. ovis*, (E) *T. pisiformis* and in untreated sheep (control). F,G: 2500 eggs of *T. hydatigena* in sheep immunized with viable embryos of *T. pisiformis* (F) on a single occasion, (G) on 12 occasions and in untreated sheep (control): H: 2500 eggs of *T. ovis* in sheep immunized with viable embryos of *T. pisiformis* on 6 occasions and in untreated sheep (control).

following three treatments with eggs of *T. hydatigena*. Similar results were obtained by feeding the eggs of *T. hydatigena* to rabbits on alternate days for 2 weeks. However, there was no effect when *T. ovis* was used instead of *T. hydatigena* (Rickard, unpublished). It seems that if a "foreign" taeniid species invades the tissues in sufficient numbers, it may induce some resistance to a normal species.

2. *Induced immunity*

In a series of experiments, lambs were injected with activated embryos of *T. pisiformis* on one or more occasions. They were then challenged 3 months later with the eggs of *T. hydatigena*, *T. ovis* or *E. granulosus* (Gemmell, 1964a, 1965b, 1966, 1969a). Class frequency data are illustrated in Fig. 5.

The total and viable cyst counts of *E. granulosus*, *T. hydatigena* and *T. ovis* were not affected by a single injection of *T. pisiformis*. When six injections of *T. pisiformis* were given, no significant effects were observed on *T. hydatigena*. When 12 injections of *T. pisiformis* were given, the total, but not the viable cyst counts of *T. hydatigena* were significantly reduced. In the case of *T. ovis*, there was a significant ($P < 0{\cdot}05$) reduction in both the total and viable cyst counts in sheep injected on six occasions with *T. pisiformis*. Similar results were obtained with the reciprocal infections in rabbits (Gemmell, 1965b). Weak immunity was induced by a single injection of embryos of *T. hydatigena* and *T. ovis* against *T. pisiformis* (data not illustrated).

No evidence was found suggesting that the reciprocal effects were as important as species-specific regulation in modifying the infective pattern.

3. *Excretory/Secretory (ES) antigens*

Evidence for the involvement of ES antigens has been obtained by implanting embryos of *T. hydatigena* and *T. ovis* in diffusion chambers into rabbits (Rickard, unpublished). The total counts of *T. pisiformis* were reduced in the former but not in the latter. Similar ES antigens may be expressed by "foreign" and normal organisms in the same intermediate host.

## VII. Maternal Factors Modifying the Infective Pattern in Homologous and Heterologous Systems

There are difficulties in interpreting studies on the maternal transfer of immunity. This is because the duration of the immune state and the persistence of protective antibody in the absence of stimulation by the ingestion of further eggs, are unknown.

Experiments with *T. saginata* in naturally immune cattle failed to detect lethal effects on larvae from serum (Froyd, 1964c) or colostrum (Urquhart, 1958, 1961). More recently, Lloyd and Soulsby (unpublished) have reported that calves can be partially protected against *T. saginata* by feeding hyperimmune serum or hyperimmune colostrum. The latter was obtained by an injection of embryos into the mammary gland. Cattle and sheep have syndesmochorial placentae and transfer of antibody to the foetus is not expected to occur

(Brambell, 1966). Any maternal transfer of antibody would be expected to take place *via* colostrum.

### A. SERUM TRANSFER OF IMMUNITY

Lambs at 2 months of age were given two injections (separated by 3 months) of activated embryos of *T. hydatigena* or *T. ovis* (Blundell *et al.*, 1968). Other lambs remained untreated. One month after the last treatment, serum from the injected and non-injected lambs was transferred intravenously to previously uninfected recipients. These were then fed with 2500 eggs of *T. hydatigena* or *T. ovis*. Class frequency data are illustrated in Fig. 6.

Significantly fewer ($P < 0{\cdot}05$) total cysts of *T. hydatigena* were present in the lambs receiving serum from lambs injected with either homologous or heterologous embryos. A degree of protection was apparent for *T. ovis* in the homologous system, but the effect was not significant in the heterologous system. No significant effects were detected against the survival rates of cysts in either system for *T. hydatigena* or *T. ovis*. Lethal effects may be conferred by circulating antibody on embryos during early reorganization into the bladder form.

### B. *IN UTERO* INFECTIONS

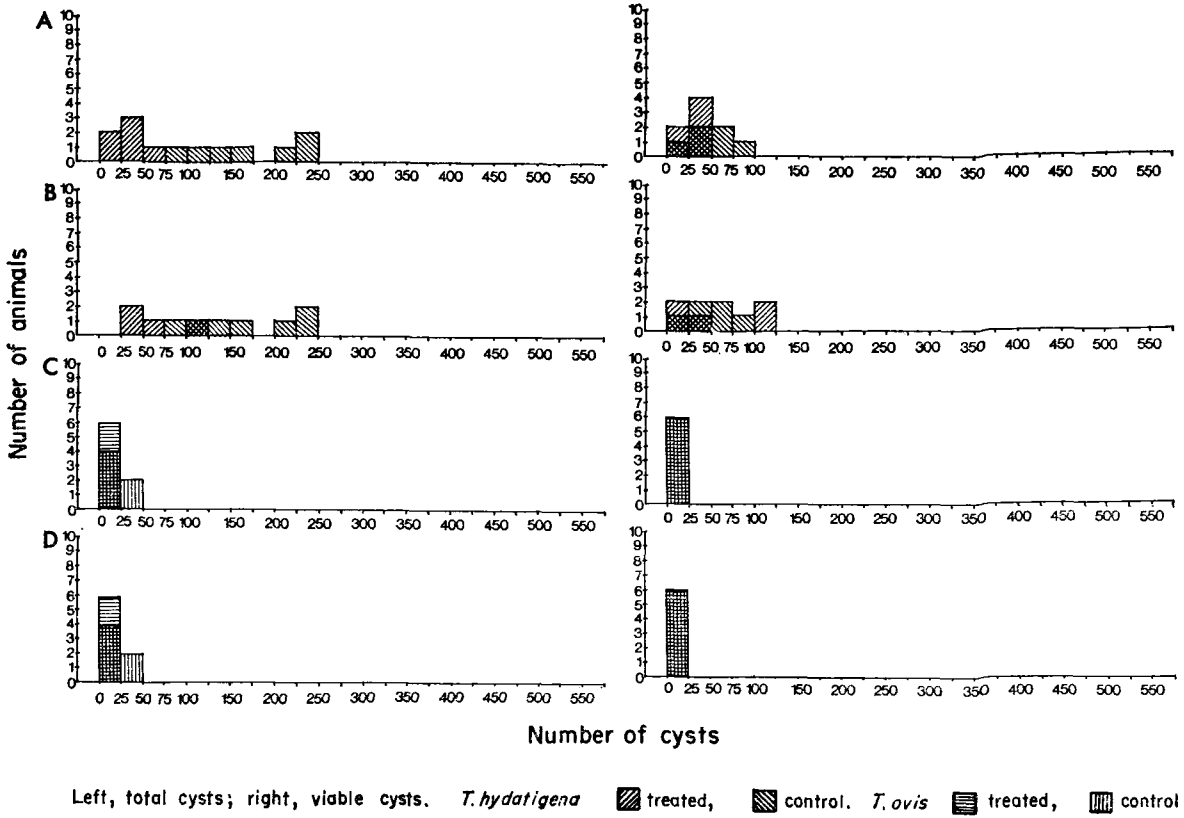
*Echinococcus granulosus* has been reported in a new-born lamb (Oparin, 1958) and a calf (Gluhovschi *et al.*, 1970). Similarly, *T. saginata* has been reported, though infrequently, in very young calves and in the bovine foetus (Ginsberg, 1958; McManus, 1960, 1963). Urquhart (1961) was unable to establish prenatal infections by feeding or injecting the eggs of *T. saginata* to naturally immune cattle. Tyshkevich (1972) was unable to infect piglets *in utero* by feeding *T. solium* although the sows became heavily infected.

An analogy to this system might be found in implantation studies using *T. hydatigena* in chick embryos (Parmeter and Gemmell, 1974). Here, organisms injected into chick embryos survived until about the 11th day after hatching. Subsequently they died, presumably destroyed by the host's defence mechanisms.

An experiment was undertaken to determine whether embryos could infect the foetus in naturally immune ewes (Gemmell, 1973a). The eggs or activated embryos of *T. ovis* were fed or injected intravenously respectively during the 8th, 5th and 3rd week before parturition. The eggs and embryos were given by the same routes to uninfected lambs. Between birth and autopsy, the lambs were retained in a tapeworm-free environment. At autopsy there was a virtual absence of *T. ovis* in the ewes and none in their lambs. It seems that embryo invasion into the foetus did not take place in the immune ewes. We have been unable to find reports of successful invasion in non-immune mothers.

### C. TRANSFER OF IMMUNITY BY COLOSTRUM

The transfer of immunity by ewes to their lambs has been assessed in two experiments. The first, using *T. hydatigena*, was concerned with defining the


A
B
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Number of animals
0 25 50 75 100 150 200 250 300 350 400 450 500 550
Number of cysts
Left, total cysts; right, viable cysts. T. hydatigena treated, control. T. ovis treated, control

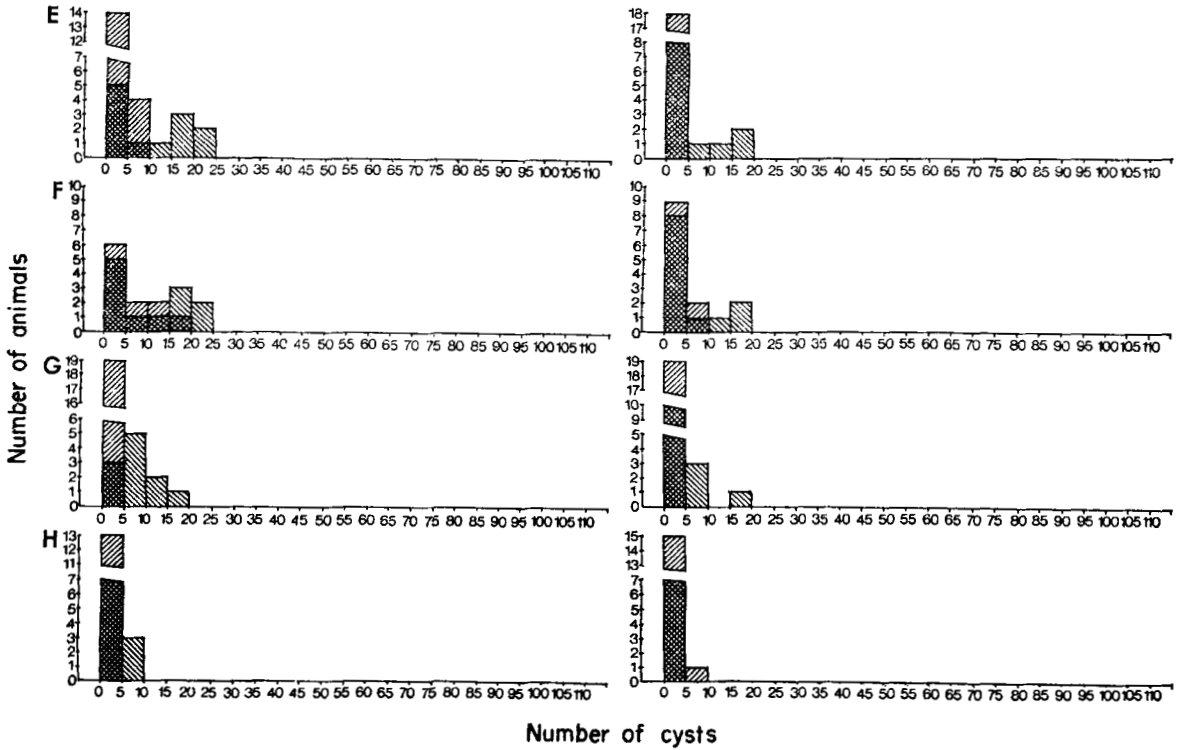



FIG. 6. Class frequency distribution of larval cestodes in sheep. A,B: 2500 eggs of *T. hydatigena* in sheep receiving serum from donors artificially immunized with embryos of (A) *T. hydatigena*, (B) *T. ovis* and from non-immunized donor sheep (control). C,D: 2500 eggs of *T. ovis* in sheep receiving serum from donors artificially immunized with embryos of (C) *T. ovis*, (D) *T. hydatigena* and from non-immunized donor sheep (control). E,F: 100 eggs of *T. hydatigena* fed to 3-day-old lambs receiving colostrum from (E) artificially immunized, (F) naturally immunized ewes and to colostrum-deprived lambs (control). G,H: 100 eggs of *T. hydatigena* fed to (G) 1-month-old, (H) 3-month-old lambs receiving colostrum from artificially immunized and naturally immunized ewes (control).

duration of the immunity following injection of embryos into the ewe (Gemmell *et al.*, 1969). The second, using *T. ovis*, attempted to demonstrate the involvement of ES antigens (Rickard and Arundel, 1974).

In the first experiment, Romney ewes were considered to be naturally immune. The immunity of one group was boosted by an injection of activated embryos of *T. hydatigena* and the remaining two groups remained untreated. The ewes from the injected group and one of the naturally immune groups were allowed to suckle their lambs. The third groups of lambs was deprived of colostrum. The lambs in each group were challenged with 100 eggs at 3 days, 1 month or 3 months of age. Autopsy was carried out 3 months later. Class frequency data are illustrated in Fig. 6.

When compared with the total cyst counts from the eggs fed at 3 days to the colostrum-deprived lambs, the counts from the injected, but not from the naturally immune animals, were significantly ($P < 0{\cdot}05$) reduced. In both these groups, however, the number but not the proportions of viable cysts were also significantly reduced. At 1 month, but not at 3 months, the total and viable cyst counts were significantly lower in the injected than in the naturally immune groups.

In the second experiment Merino ewes of one group were injected at 6 and 2 weeks prior to lambing with antigen obtained from cultured *T. ovis* in Freund's Incomplete Adjuvant. The other two groups were considered to be naturally immune. Lambs from these latter two groups were either suckled or deprived of colostrum. At 2 and 8 weeks after birth, the lambs of all three groups were challenged with 1500 eggs and autopsied. It seemed that *T. ovis* developed in the colostrum-deprived group only (data not illustrated).

Both experiments indicated the transfer of some immunity from injected ewes to their offspring. They are, however, in conflict with respect to the transfer of immunity from the naturally immune ewes. Unfortunately, previously uninfected ewes were not available and absorption studies were not undertaken to determine the specificity of the transferred immunity. Explanations involving differences in breeds of sheep, species of tapeworm or in the level of immunity of the dams, have still to be resolved. Verster (1969) points out that *T. ovis* may not have evolved as a parasite of sheep. This, together with other observations, suggests that *T. ovis* is not as well adapted as *T. hydatigena* to survive in sheep. It also emphasizes the point that identical host–parasite relationships among larval cestodes cannot be taken for granted.

## VIII. Observations on Egg Populations and their Effective Life Span

The process of hatching of taeniid eggs has been described by many authors (Isobé, 1922; Ross, 1929; Yoshino, 1933a,b, 1934; Bullock *et al.*, 1934; Penfold *et al.*, 1937a; Leonard and Leonard, 1941; Silverman, 1954a,b; Berberian, 1957; Meyers, 1957; Jones *et al.*, 1960; Meymerian, 1961; Parnell, 1965; Laws, 1967, 1968a,b; Gönnert *et al.*, 1967; Gönnert and Thomas, 1969; Gönnert, 1970; Burkhardt and Dwaronat, 1970; Heath and Smyth, 1970; Gemmell, 1976a). Post-embryonic development has been reviewed by Voge (1967).

While there are species differences in the chemical requirements for hatching, the sequence of events which occurs is similar. This includes the disaggregation of the embryophoral blocks and disintegration of the basal membranes followed by mechanical and possibly chemical disruption of the oncospheral membrane with release of the embryo.

### A. MATURATION OF EGGS

Several workers have referred to an association between immaturity and the failure of eggs to hatch successfully (Silverman, 1954a,b; Berberian, 1957; Meymerian, 1961; Huffman and Jones, 1962; Gemmell, 1976a). The hatchability of eggs varies between worms of the same species and also between segments. Furthermore, the duration of motility also varies widely between individual organisms. Based on the premise that *in vitro* observations more or less simulate *in vivo* events, Gemmell (1976a) proposed that in each segment there is a number of populations of eggs, morphologically similar, consisting of juvenile, competent and senescent individuals. The ratio of each population in each segment is independent of the order on the strobila.

### B. LONGEVITY OF EGGS

Eggs of *T. saginata* maintained under a variety of natural and artificial environments may live for several months (Penfold *et al.*, 1936b, 1937b; Duthy and van Someren, 1948; Jepsen and Roth, 1952; Silverman, 1956; Lucker and Douvres, 1960; Froyd, 1962; Suvorov, 1965; Enigk *et al.*, 1969; Nadzhafov and Chobanov, 1973). Long-term survival in nature has also been reported for *Echinococcus* (Thomas and Babero, 1956; Sweatman and Williams, 1963; Vibe, 1968) and for *T. hydatigena* (Shepelev, 1961a; Sweatman and Williams, 1963).

### C. FACTORS LETHAL TO EGGS

Factors affecting the survival of eggs have been reviewed in Gemmell (1976d). High temperatures are inimical to survival (Nosik, 1952; Silverman, 1956; Meymerian and Schwabe, 1962; Williams, 1963; Enigk *et al.*, 1969; Williams and Colli, 1970; Colli and Williams, 1972; Gemmell, 1976a). Desiccation is also lethal (Penfold *et al.*, 1937b; Jepsen and Roth, 1952; Silverman, 1956; Lucker and Douvres, 1960; Laws, 1968a; Gemmell, 1976a). In addition, microbial contaminants have been implicated (Silverman, 1956; Laws, 1967, 1968b; Gemmell, 1976a).

### D. FACTORS MODIFYING THE LIFE SPAN OF EGGS

The maturation of eggs outside the proglottid (Silverman, 1954b) appears to be temperature-dependent (Gemmell, 1976a). Taeniid eggs may survive for long periods at temperatures below 38°C. The eggs of *Echinococcus* (Dévé, 1910; Schiller, 1955; Lukashenko, 1962; Colli and Williams, 1972), *T. saginata*

(Lucker, 1960; Suvorov, 1965) and *T. hydatigena* and *T. ovis* (Gemmell, 1976a) may survive in sub-zero temperatures.

Ultraviolet irradiation appears to reduce the infectivity; storage may modify the susceptibility to X-irradiation of eggs of *E. granulosus* (Williams and Colli, 1972). Based on *in vitro* studies with *T. hydatigena* and *T. ovis*, Gemmell (1976a) concluded that temperatures between 7°C and 38°C accelerated the ageing process of competent organisms and stimulated a proportion of juveniles to replace them, whereas sub-zero temperatures limited replacements without significantly retarding the ageing process.

### E. PENETRATION OF TISSUES BY AGEING ORGANISMS

Batham (1957) observed that the eggs of *E. granulosus* stored at up to 32°C for between 10 and 21 days, developed into cysts in mice. After longer periods invasion without survival occurred. Eggs of *T. hydatigena* stored in water at 7°C for 90 days developed into cysts in sheep. Some stored for 273 days were still invasive, however, but died out before reorganization had been completed (Gemmell, 1976a). While the absence of cysts does not imply that senescent embryos failed to penetrate the tissues, the positive results are consistent with the hypothesis that some senescent organisms can invade the tissues without completing their reorganization.

Such an hypothesis is consistent with the concept that less than full competence prevents embryos rapidly reaching an antibody-resistant phase before effective amounts of antibody are produced in the "naïve" animal (Rickard, 1974). Abnormalities in the growth and development of embryos of *T. pisiformis* have been observed following X-irradiation (Beveridge and Rickard, 1975). The epidemiological implication is that if senescent organisms are ingested first giving rise to an abortive infection (Gemmell, 1975), they should induce resistance without infection in the same way as observed for irradiated eggs of *T. taeniaeformis* by Dow *et al.* (1962). This has also been reported to occur for *T. saginata* in cattle (Wikerhauser *et al.*, 1974).

## IX. Infection Pressures and Dispersion Patterns of Eggs in the Field

Serodiagnosis of larval cestodes in sheep (Blundell-Hasell, 1969; Kagan, 1974), cattle (Pawlowski and Schultz, 1972) and pigs (Proctor and Elsdon-Dew, 1966; Kagan, 1974) has not reached the stage where it can be used as a tool in epidemiology. There is no problem in detecting specific antibody in heavily parasitized animals, but the specificity of the tests with naturally infected animals is low and cross-reactivity is high.

Outbreaks of cysticercosis in cattle have been studied by Dewhirst *et al.* (1967) and Schultz *et al.* (1969); outbreaks of cysticercosis in man by Heinz and Macnab (1965) and Heinz and Klintworth (1965). Gemmell (1968) described changes in the infective pattern of ovine hydatidosis and cysticercosis during a control programme. Schantz *et al.* (1970) investigated transmission of *E. granulosus* on a farm basis.

Estimations of the infection pressure and indices of clustering of eggs may

help to explain the events that give rise to the observed incidence and prevalence rates. Schwabe (1964) described the "incidence rate" of a disease as a dynamic measure of the number of new cases occurring in a given population in a stated period of time, and the "prevalence rate" of a disease as a static measure of the total number of affected individuals in a population at a given time. If eggs are picked up singly and at random by susceptible animals, the number of cysts that develop should be distributed as a Poisson variate. A test of this distribution is provided by the Index of Dispersion $\Sigma(x - \bar{x})^2/\bar{x}$ (Fisher, 1954). If eggs are randomly ingested in clusters of any size, the proportion of animals that escape infection is given by $e^{-kt}$; where $k$ is a constant called the infection pressure, $t$ is the duration of exposure, and $e$ is the base of the natural logarithms. Thus, the proportion of animals that have become infected after time $t$ is given by the equation $Y = 1 - e^{-kt}$.

The problem with identifying the dispersion pattern of eggs from cyst counts is complicated by the variability of the innate resistance (see Table I) within the host population. If the period of exposure is short enough, further modifying effects of acquired immunity should not be important.

### A. PRESENCE OR ABSENCE OF DEFINITIVE HOST

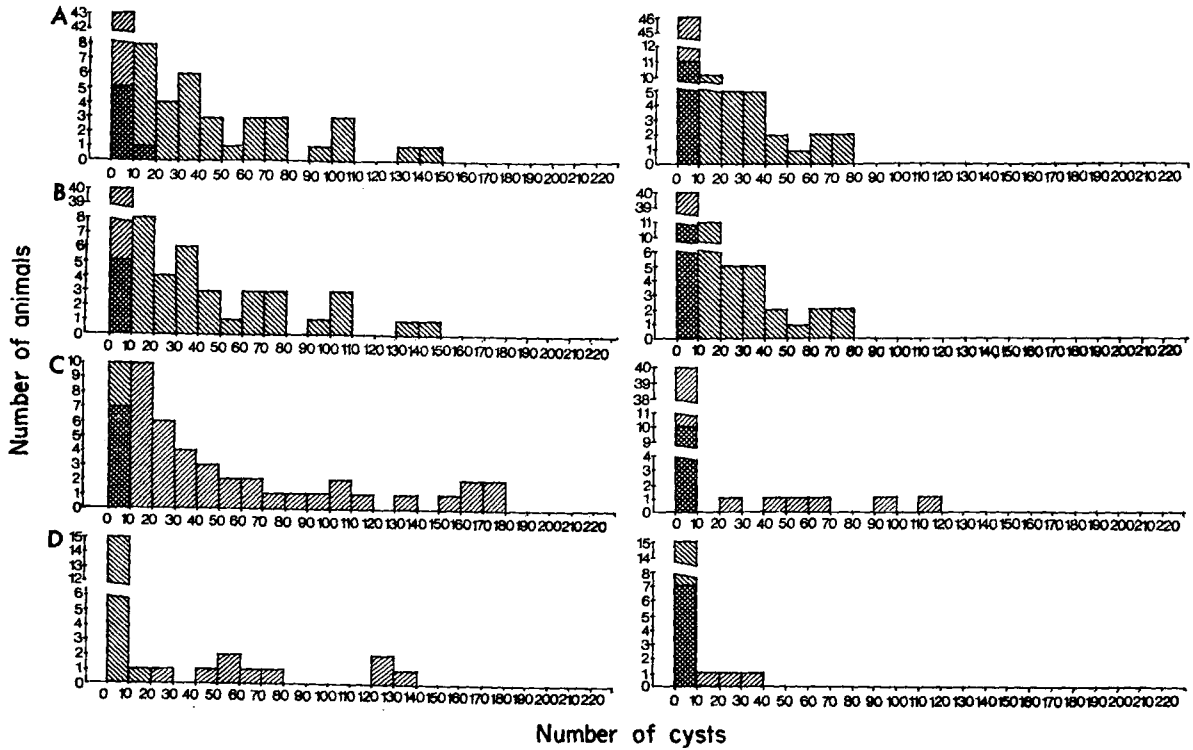
A brief description is required of the potential output of eggs from the tapeworm in the definitive host. Tapeworms such as *T. saginata* shed enormous numbers of eggs. Penfold *et al.* (1937c) estimated that a proglottid contained $80 \times 10^3$ eggs and the estimated mean daily output of eggs, although highly variable, was $720 \times 10^3$. The daily output of proglottids has been estimated at between six and nine (Penfold *et al.*, 1937c; Belyaev and Monisov, 1967). Individual daily extremes of up to 40 segments have been recorded (Bonilla-Naar, 1946). Not all segments contain a full complement of ripe eggs (Rijpstra *et al.*, 1961). Five segments of *T. solium* each containing between $50 \times 10^3$ and $60 \times 10^3$ eggs may be extruded daily (Webbe, 1967). The mean number of eggs of *T. hydatigena* per proglottid has been estimated at $28 \times 10^3$ and an average of two segments may be shed daily (Featherston, 1969). Higher mean egg counts have been recorded for *T. ovis* (Arundel, 1972). These estimates demonstrate the biotic potential but provide little information on the proportion that become available to the intermediate host.

#### 1. *Definitive host present*

Eggs initially will be deposited in clusters in and around the faecal mass, or near segments expelled independently. Subsequently, they are dispersed.

An experiment was undertaken to estimate the infection pressure from eggs and clustering effect of eggs that might be expected when dogs with long-term infections of *T. hydatigena* are retained in an endemic zone (Gemmell and Macnamara, 1976). Five months after eight dogs with *T. hydatigena* were first placed on a pasture, susceptible lambs were grazed there for 4, 8, 16 or 32 days with the dogs still present. The lambs were then returned to the tapeworm-free environment and autopsied 3 months later.

The class frequency data for all grazing periods have been combined and are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Number of animals
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Left, total cysts; right, viable cysts. T. hydatigena treated, control.

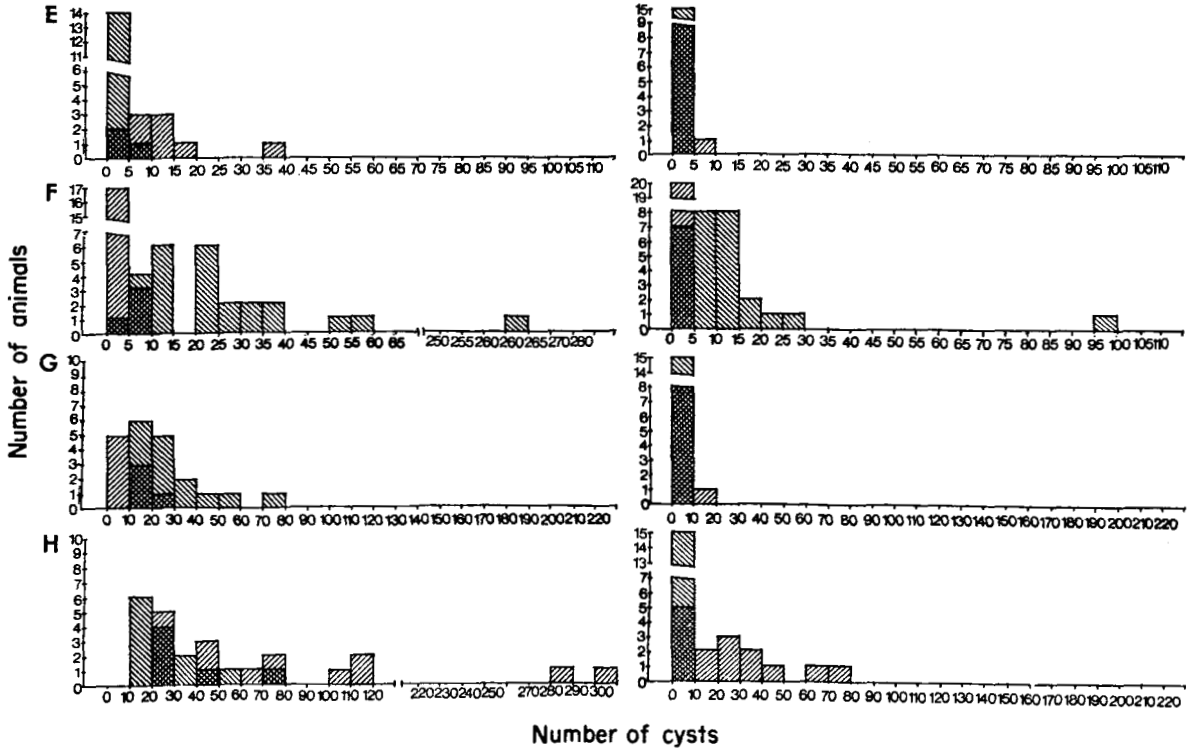



FIG. 7. Class frequency distribution of larval cestodes in sheep. A,B: Cyst counts in lambs grazed for up to 32 days on pasture from which infected dogs absent for (A) 3 months, (B) 6 months and in lambs grazed on pasture when dogs present (control). C: Cyst counts in lambs grazed for 10-day periods after infected dogs introduced and in lambs grazed on pasture before the introduction (control). D,E: Cyst counts in lambs grazed for 10-day periods within (D) 0–25 m, (E) 25–80 m of infected dog and in lambs grazed on the pasture before the introduction of the dog (control). F: Cyst counts in 0 to 3-week-old and 5 to 12-week-old (control) lambs grazed for 7 days on pasture in presence of infected dogs. G,H: Cyst counts in lambs grazed from (G) 0–3 months, (H) 3–6 months and in lambs grazed from 0–6 months (control) on pasture contaminated with eggs.

illustrated in Fig. 7. Within 4 days of grazing the pasture, all lambs had ingested eggs. Each day about 60% of the flock ingested eggs. Irrespective of the grazing period, the distribution of the cyst counts showed a marked departure from the Poisson model. These results emphasize the short grazing period required before the herd or flock becomes infected.

2. *Definitive host removed*

The equilibrium between the gain and loss of eggs will be disturbed and the ratio of competent to senescent organisms should alter as soon as the definitive host is treated successfully or has been removed. Three and 6 months after the dogs had been removed, lambs were placed on pasture for similar periods as described previously. Within about 3 and 6 months of the removal of the dogs, the infection pressure had been reduced to 6·5% and 3·5% respectively. After 3 months, the Index of Dispersion for the number of cysts present in the lambs that had grazed the pasture for 4 and 32 days was significantly different from a Poisson distribution. After 6 months, the differences were still significant in the lambs that had grazed for 4 and 16 days. Thus, complete dispersion of some clusters of eggs may take more than 6 months from the time of deposition. There was also a continuing reduction in the survival rates of the cysts during this period. This provides some field evidence for a decline in the ratio of competent to senescent organisms.

3. *Definitive host introduced*

The rapidity of the build-up of eggs was also measured from cyst counts obtained in sheep grazed near to two dogs each infected with four egg-laying *T. hydatigena* (Gemmell, 1976b). Groups of lambs were grazed on the pasture for 10-day periods up to 85 days after the dogs had been introduced. The class frequency data have been combined and are illustrated in Fig. 7. Before the dogs were introduced, only a small number of randomly dispersed "wild" eggs were available to the sheep. Within 10 days of introducing the dogs, a striking increase in the mean cyst counts was apparent. These remained at a high level during the 85 days of the experiment. These results emphasize the rapidity with which a pasture can become virtually saturated with eggs.

### B. EGG DISPERSION

The question arises as to whether the majority of eggs remain near the faeces or disperse, or whether the defaecation habits of the definitive host are solely responsible for the scatter. Before these and similar questions can be answered, estimates have to be made of the egg dispersion pattern under various climatic and ecological situations.

1. *Egg spread on pasture*

Sweatman and Williams (1963) sprayed eggs of *E. granulosus* and *T. hydatigena* onto pastures in two climatic zones. In an oceanic-type climate with an annual rainfall of 3000 mm, the eggs remained available to sheep throughout the 4-month winter period, but were subsequently carried deep into

the soil. Sweatman and Williams suggested that high rainfall was an important factor in limiting the availability of eggs. In the zone with a continental-type climate and an annual rainfall of only 80 mm, the eggs were also available during the 4-month winter period, but following a hot dry summer, they disappeared from the site of deposition. It was considered that the eggs had drifted in wind currents.

Estimates have been made of the dispersion of eggs (Gemmell and Johnstone, 1976). Lambs grazed for 10 days before, as well as 10 and 40 days after, one dog with four patent *T. hydatigena* had been introduced. Class frequency data on the cyst counts obtained in sheep grazed at various distances before and after the dog had been introduced are illustrated in Fig. 7. A few dispersed "wild" eggs were present on each plot before the introduction of the dog. Afterwards, there was a massive build-up of eggs within 25 m of the kennel with some spread up to 80 m. No spread of eggs was detected beyond that point. These estimates refer only to the situation where there is an adequate grass cover and a natural deposition of eggs at a specific site.

Perhaps the most striking observation on contamination of the environment with taeniid eggs was that recorded by Schwabe (1964). In a systematic examination of street dirt in slum areas of Beirut, between 25 % and 36 % of the samples examined contained taeniid eggs. Further, salad greens were also contaminated with eggs.

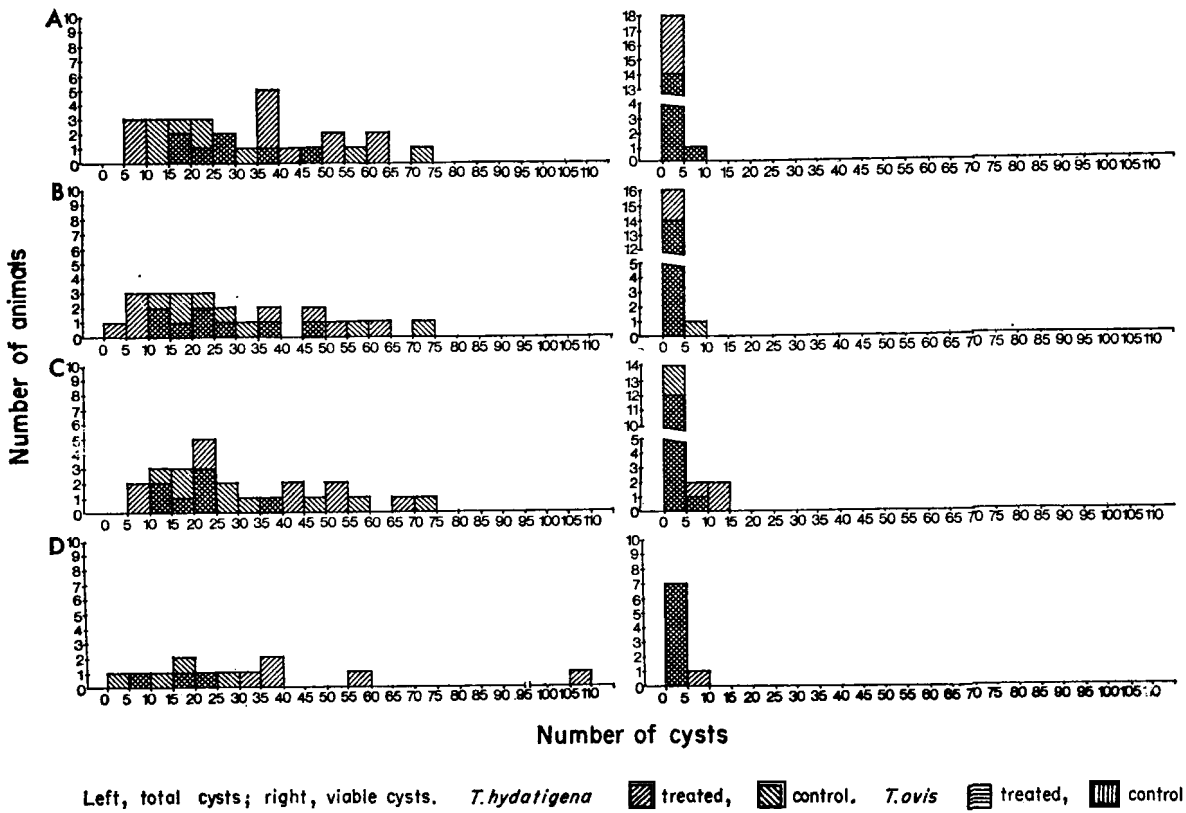
2. *Transportation of eggs*

Transportation of definitive hosts facilitates the spread of eggs. For example, it has been suggested that the eggs of *T. saginata* can be spread from lavatories on trains (Anon., 1954). The increased popularity of camping and use of imperfectly purified effluents as fertilizer have been suggested as causes for the spread of *T. saginata* (Pellegrini, 1964).

Some eggs will survive most conventional sewer systems. Sewage discharge with overflow onto pastures or directly into rivers and their estuaries is likely to disperse eggs (Profé, 1934; Vasilkova, 1944; Newton *et al.*, 1949; Wang and Dunlop, 1954; Silverman, 1955b; Sinnecker, 1955, 1958; Miller, 1956; Greenberg and Dean, 1958; Kabler, 1959; Silverman and Guiver, 1960; Liebmann, 1963; Menschel, 1964; Denecke, 1966; Amirov and Salamov, 1967). Eggs may be dispersed by birds (Götzsche, 1951; Silverman and Griffiths, 1955a,b; Guildal, 1956; Crewe, 1967; Crewe and Crewe, 1969).

Invertebrates such as earthworms, cockroaches and dung beetles are important in the life-cycle of families such as Hymenolepididae and may be involved in the transport of taeniid eggs.

Filth flies are capable of carrying tapeworm eggs for several days externally and internally (Nicoll, 1911; Shircore, 1916; Sycevskaja and Petrova, 1958; Round, 1961; Nadzafov, 1967). Flies have been shown to transfer hydatid eggs to rodents (Schiller, 1954) and they may transfer eggs to human food (Heinz and Brauns, 1955). Tagging of flies has shown a wide dispersion pattern (Linquist *et al.*, 1951; Yates *et al.*, 1952; Schoof *et al.*, 1952; Schoof and Siverly 1954a,b). Biting flies are unlikely to transmit eggs of *T. saginata* directly to cattle (Urquhart, 1965).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Left, total cysts; right, viable cysts. T. hydatigena treated, control. T. ovis treated, control

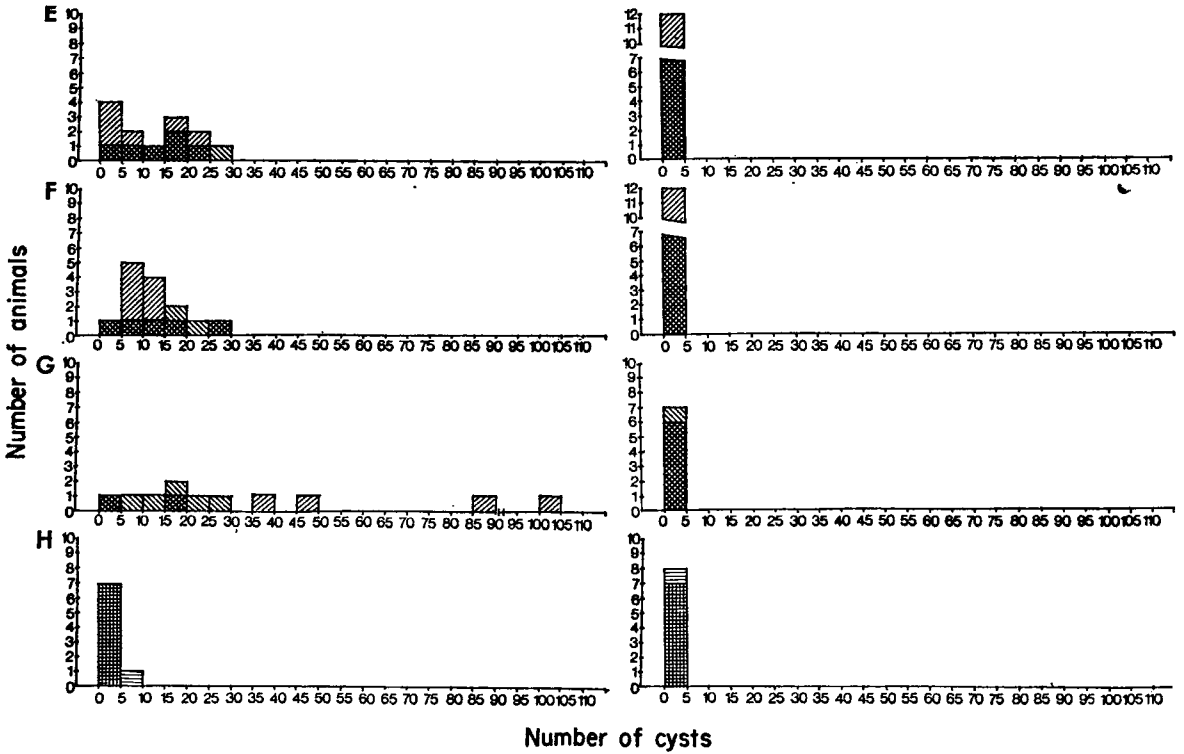



FIG. 8. Class frequency distribution of larval cestodes in sheep. A,B,C: Cyst counts in 6-month-old lambs (A) receiving colostrum from hyper-immunized ewes, (B) actively immunized at 1 week, (C) actively immunized at 3 months and in similar lambs (control) also reared on pasture contaminated with the eggs of *T. hydatigena*. D,E,F,G: Cyst counts in 9-month-old pasture-reared lambs (D) at the site of predilection from natural infection and specific challenge with 2500 eggs at 6 months, (E) at the site of injection from homologous embryos implanted at 3 months, (F,G) at the site of predilection from natural infection and specific challenge with eggs at 6 months in lambs immunized at 3 months with (F) homologous embryos, (G) heterologous embryos and in similar lambs (control) also reared from birth on pasture contaminated with the eggs of *T. hydatigena*. H: Cyst counts of *T. ovis* in 9-month-old lambs at the site of predilection from a specific challenge of 2500 eggs of *T. hydatigena* and *T. ovis* at 6 months and in similar untreated lambs (control) also reared on pasture contaminated with eggs of *T. hydatigena* (experiment continued in Fig. 9 A,H).

We have been unable, however, to find studies that measure the extent of the transport of eggs by individual systems or of their practical importance in determining the infective pattern of cysticercosis and hydatidosis.

## X. Animal Husbandry Practices Modifying the Infective Pattern

### A. Rearing livestock from birth under a high infection pressure

Three experiments have been selected for review. These explore the effect of the infective pattern of rearing animals from birth under various infection pressures and egg-clustering situations. Class frequency data are illustrated in Figs 7 and 8.

In the first experiment, lambs of specific ages between 0 and 12 weeks were grazed for 7-day periods on a pasture near dogs infected with *T. hydatigena* (Gemmell, 1976c). The total and viable counts as well as the proportions surviving were significantly lower ($P < 0{\cdot}01$) in the groups of lambs grazed for one week during the first 3 weeks after birth than in those grazed for the same period from 5 to 12 weeks of age. The high total cyst counts coincided with the change from a predominantly sucking to a predominantly grazing animals.

In the second experiment, lambs were grazed from birth for 3 or 6 months or were introduced at 3 months of age onto a pasture heavily contaminated with the eggs of *T. hydatigena* (Gemmell, 1972a). The total cyst counts were significantly ($P < 0{\cdot}01$) lower in the lambs grazed from birth to 3 months than from birth to 6 months. These in turn were much lower than that in those introduced onto the pasture at 3 months. The proportion of cysts surviving was lowest in the group grazed during the whole 6-month period. While the invasion of embryos was not prevented altogether between 3 and 6 months, few survived. Such results might be expected if the lambs reared from birth on the pasture gradually acquired strong resistance due to the continued invasion of embryos.

In the third experiment, lambs from hyperimmunized or naturally immune ewes were grazed from birth for 6 months on pasture near dogs infected with *T. hydatigena* (Gemmell, 1972b). The lambs from the naturally immune ewes were either artificially immunized at one week or 3 months of age or remained untreated. There were no significant differences in the total cyst counts between any of the groups of lambs. The viable cyst counts in the lambs from the hyperimmunized ewes did not differ from those from the naturally immunized ewes. In contrast, the viable cyst count in the animals immunized at one week of age was significantly lower ($P < 0{\cdot}05$) than those in all other groups. Such results might be expected if the transfer of immunity via colostrum had no long-term effect and if active immunization at one week of age failed to prevent continued embryo invasion, but induced an immune response to their survival.

### B. Introduction of definitive hosts with homologous and heterologous infections into endemic zones

In a field immunization experiment, eggs of *T. hydatigena* and *T. ovis*

were fed or injected into lambs reared on a pasture contaminated with the eggs of *T. hydatigena* (Gemmell, 1970b). Since the eggs were introduced orally or parenterally, it is possible to distinguish between the cysts from the eggs introduced by injection at 3 months from those fed at 6 months. Class frequency data are illustrated in Figs 8 and 9.

The results obtained indicated that homologous embryos introduced at 3 months developed without surviving and boosted the immunity to the resident taeniid species. The heterologous embryos developed but did not modify the infective pattern of the resident species. The simultaneous introduction of two species at 6 months induced a non-specific lethal effect modifying the infective pattern of the heterologous species. Clearly, a number of experiments are required to demonstrate interactions between species in the field situation.

### C. INTRODUCTION OF NATURALLY AND ARTIFICALLY IMMUNIZED INTERMEDIATE HOSTS ONTO PASTURES WITH VARIOUS EGG-CLUSTERING PATTERNS

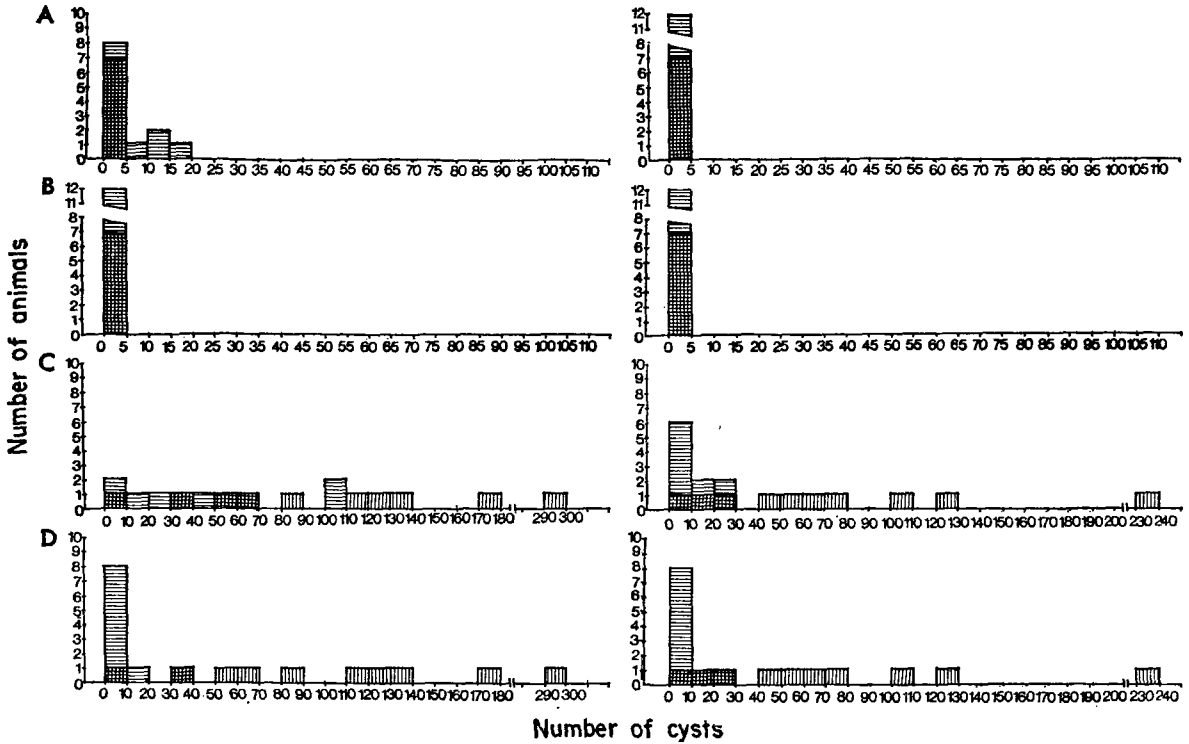
In the experiment described here, the pasture was divided into inner, middle and outer zones (Rickard *et al.*, 1975). Two dogs infected with *T. ovis* were placed in the inner zone for 5 weeks. Groups of lambs previously exposed to infection, together with lambs injected with antigen from a 14-day culture of *T. ovis* (Rickard and Bell, 1971a,b,c), and control lambs, were introduced into each zone for 6 or more weeks. The previously infected lambs had been allowed to graze on pasture naturally contaminated with the eggs of *T. ovis* 5 months before being used in the trial. Class frequency data are illustrated in Fig. 9.

The aggregation effects observed in the cyst counts of the controls may be regarded as a sign of egg clustering in all three grazing zones. They suggest that the inner zone, in particular, was virtually saturated with eggs. The total cyst counts were significantly lower ($P < 0{\cdot}01$) in the vaccinates than in any other group in all zones. The counts in naturally exposed lambs did not differ from the controls in any zone. The proportion of cysts surviving was significantly lower ($P < 0{\cdot}01$) in the previously naturally exposed animals than in the vaccinates or controls. Thus, the vaccinated lambs were able to resist infection better than the other groups, but only the previously naturally exposed lambs were able to mount a rapid response to kill the cysts during reorganization.

It seems that the pattern of the egg distribution may modify the infective pattern both with regard to the number that invade and the proportion that reach the infective stage in young animals that have previously been exposed to infection. The duration of immunity may assume importance in ecological situations where eggs die off or become unavailable for long periods. This highlights the need to define the duration of the various immunities in the absence of continuous impinging infections.

### D. DEVELOPMENT OF THE "CYSTICERCOSIS STORM"

Several experiments have already been described recording very high cyst counts when susceptible sheep are introduced onto pasture virtually saturated




Left, total cysts; right, viable cysts. *T. ovis* treated, control.

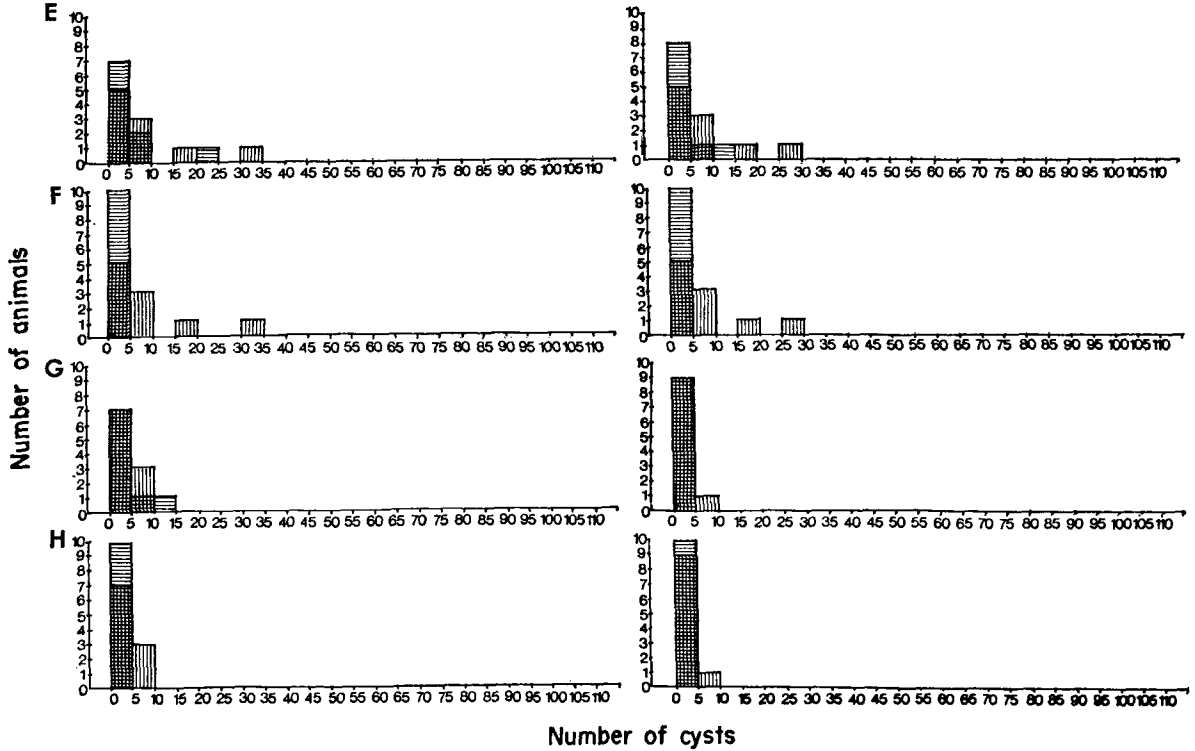



FIG. 9. Class frequency distribution of larval cestodes in sheep. A,B: Cyst counts in 9-month-old pasture-reared lambs (A) at the site of injection from the implantation of embryos at 3 months, (B) at the site of predilection from (C) a specific challenge of 2500 eggs of both *T. ovis* and *T. hydatigena* at 6 months and in similar untreated lambs (control) reared on pasture contaminated with the eggs of *T. hydatigena* (continued from Fig. 8). C,E,G: Cyst counts in previously naturally exposed lambs and in untreated lambs (control) grazed for 6 weeks at varying distances (C) inner, (E) middle, (G) outer paddock from dogs infected with *T. ovis*. D,F,H: Cyst counts in vaccinated lambs and in untreated lambs (control) grazed for 6 weeks at varying distances (C) inner, (F) middle, (H) outer paddock from dogs infected with *T. ovis*.

with eggs. Storms have also been recorded for *T. saginata* in cattle maintained in feed lots (Schultz *et al.*, 1969) and in calves infected from attendants using hand-suckling techniques (Urquhart, 1961; Froyd, 1961; Goulart *et al.*, 1966). Personal habits and husbandry practices amongst certain East African tribes may also contribute to the development of heavy infections of *T. saginata* in cattle (Froyd, 1965a,b). Viljoen (1937) also described scavenging by pigs as a source of heavy infections of *T. solium*. Another method of transmission that might be placed in this category has been described for *T. solium* (Heinz and Macnab, 1965; Heinz and Klintworth, 1965). Here the use of tapeworm segments in folk medicaments might well give rise to heavy infections and epilepsy.

Under a strong infection pressure, the development of large numbers of cysts occur in the 14-day interval between the ingestion of the first eggs and the onset of immunity. Certain movements of animals prevent, whereas others assist, the outbreak of a "cysticercosis storm". Paradoxically, movements that tend to prevent high cyst counts include the raising of livestock from birth in moderately close association with infective definitive hosts. Those that tend to encourage heavy infections include the introduction of susceptible animals during the course of a control programme (Gemmell, 1968). In the latter situation, the severity of the storm will depend on the length of time the zone has been free from eggs. The longer this period, the greater will be the number of age cohorts that may become heavily parasitized.

## XI. Special Considerations in Human Hydatidosis and Cysticercosis

Considerable research has been undertaken on characterizing antigens and developing serological tests for the identification of specific clinical disease from cysticercosis (Elsdon-Dew, 1967; Rydzewski *et al.*, 1975) and from hydatidosis (Kagan, 1968, 1970, 1973, 1974; Kagan and Agosin, 1968; Capron *et al.*, 1970; Varela-Díaz and Coltorti, 1974). The potential of serodiagnosis in epidemiology has not yet been adequately explored. Methods of assessing errors from serodiagnosis of hydatidosis in populations are described in Marchevsky (1974).

Most of the factors that modify the infective pattern of larval cestode populations in domestic animals should also operate to limit these parasitoses in man. Little is known of the variability of innate resistance to hydatidosis in man. Individual clinical infection may be a matter of chance, but high indices of infection in the susceptible population may reflect low standards of hygiene in circumstances that favour effective transmission. Outstanding early reviews on the host–parasite relationship in hydatidosis include that of Krabbe (1864) in Iceland and that of Thomas (1884) in Australia. Many situations favouring successful transmission have recently been described, for example a higher prevalence than expected among shoemakers in the Lebanon (Schwabe and Daoud, (1961); a life-cycle involving sledge dogs, voles and human infections with *E. multilocularis* on St Lawrence Island (Fay, 1973); rural hydatidosis from *E. granulosus* in Uruguay (Purriel *et al.*, 1973) and among the Turkhana

in Kenya (Mann, 1974) and among the Basque population of California (Araujo *et al.*, 1975).

Experience has shown that during the control programmes in Iceland, New Zealand and Tasmania, the age-specific surgical incidence rates of hydatidosis decreased in the human population before they did in the normal animal hosts (Meldrum and McConnell, 1968; Beard, 1973a,b; Gemmell, 1973b). Changes in the relationships between infection rates in dogs, sheep and man during the educational and subsequent official control programmes directed against *E. granulosus* in Tasmania and New Zealand are illustrated in Figs 10 and 11. It

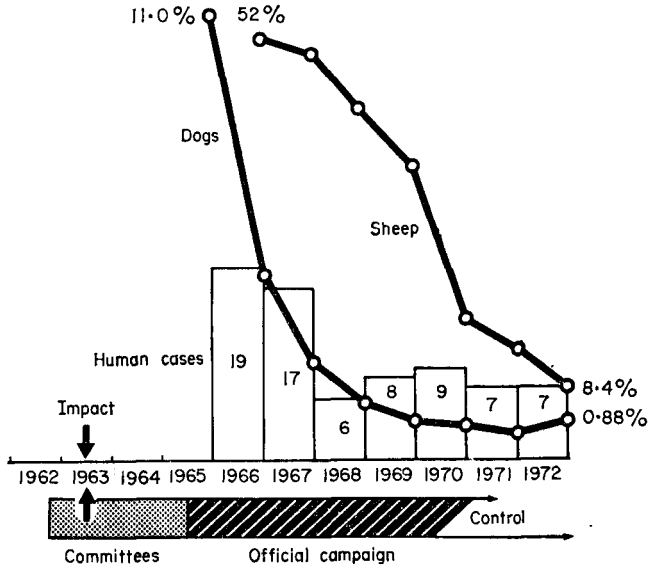



FIG. 10. Surgical cases of hydatid disease (*Echinococcus granulosus*) in man compared with prevalence in dogs and sheep, and timing of control measures in Tasmania (1962–72). (After Beard, 1973b, with permission.)

seems that in both control programmes, cases in children and adults that required surgery early after infection may form a distinct sub-group (Beard, 1973b). This sub-group was removed early during a period of rapidly falling incidence in the dog population.

Some individuals acquire heavy infections of larval *T. solium*. A search of the literature, e.g. Acha and Aguilar (1964), has failed to reveal any statitical evidence associating a greater prevalence of clinical cysticercosis in individuals parasitized with *T. solium* than in those free from this tapeworm. Heinz and Macnab (1965) found the chances of a non-pork eater becoming infected with cysticercosis to be as great as those of a pork eater. Clearly, an infected individual with wide social contacts may infect many persons.

Leuckart (1856) suggested that cysticercosis in man could occur directly by auto-infection. As pointed out by Heinz and Macnab (1965), this possibility

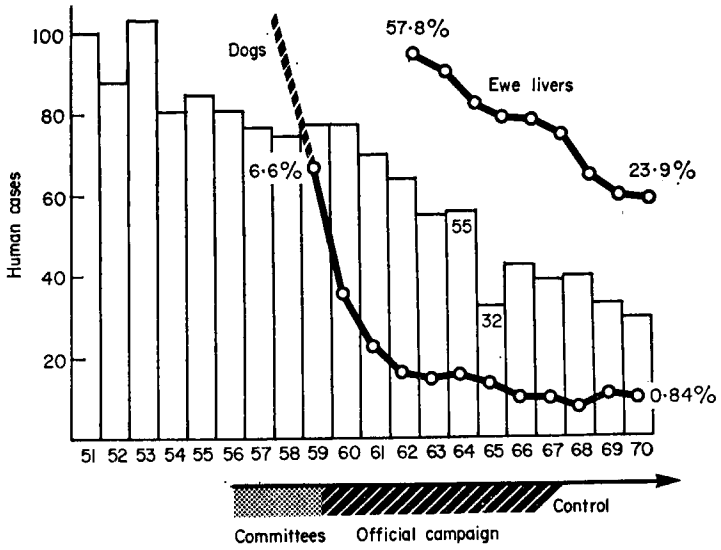



FIG. 11. Surgical cases of hydatid disease (*Echinococcus granulosus*) in man compared with prevalence in dogs and sheep, and timing of control measures in New Zealand (1951–1970). (Modified from Beard, 1973b, with permission, from data supplied by the National Health Statistics Centre, N.Z., 1975.)

has often been changed to probability in the literature. A number of workers, e.g. Jopling and Woodruff (1959), considered this sufficient reason for caution in the treatment of this tapeworm infection. Webbe (1967) concluded that the case for endogeneous auto-infection has been overestimated. The most cogent reason given was that the eggs would have to reach the stomach through reverse peristalsis before hatching could be initiated. There are other factors limiting auto-infection. Individuals that have been infected by small doses of eggs at an early age may be resistant to self-infection when a tapeworm becomes established. Those that are susceptible to auto-infection at the time of infection with the tapeworm may remain so for only 7 to 14 days after the first embryos are liberated in the intestine.

## XII. CONCLUSIONS

Almost invariably, surveys on larval cestodes in man and his domestic animals record prevalence rates of less than 100%. The "uninfected" proportion consists of individuals that have not been exposed to eggs, those that were innately resistant, and those that were susceptible but received an abortive infection from senescent eggs and acquired immunity.

The extent of innate resistance to different larval cestodes varies within intermediate-host populations. In addition, immunity to superinfection is acquired and larvae survive in only a proportion of the intermediate-host

population. Only that proportion will play any active part in the transmission of hydatidosis and cysticercosis. Before the onset of acquired resistance in the intermediate host, the infective pattern of larval cestodes will be modified by the infection pressure, the age of the host at the time of the first infection, the sequence and frequency of subsequent infections, feeding habits, livestock movements, egg-dispersion, and the invasiveness of the embryos.

Where no effective control programmes operate, the intrinsic host-operated immunities should virtually be constants in any given host population and the extrinsic factors should be the variables in the epidemiological equation. Since the period for successful infection is relatively short, it should be possible to develop experimental models to fit many of the ecological situations where larval cestodes cause health or economic problems. We believe such models would assist in planning effective control programmes.

While the high biotic potential satisfies a need for an excess of eggs for the successful adjustment of the parasite population in the intermediate host, very little is known of the factors that determine the infective pattern of taeniid tapeworms in the definitive host. Further quantitive studies on this aspect are required before the epidemiology of hydatidosis and cysticercosis can be fully understood.

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